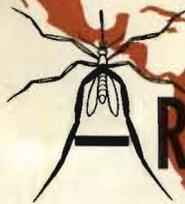


ENTOMOLOGICAL INVESTIGATIONS PROGRAM
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ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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

INTRODUCTORY NOTES FROM THE SUB-COMMITTEE ON INFORMATION EXCHANGE

The reception of the first issue of the Arthropod-borne Virus Information Exchange newsletter was enthusiastic and has prompted issue of this second number on the previously suggested schedule of three times a year - April, September and January. As originally planned, the April issue will be devoted primarily to annual report summaries, while the other two issues will contain progress reports or mention of projects planned or being implemented.

The Catalogue of Arthropod-borne Viruses, an obviously more sizeable undertaking, at least until all published viruses are represented, was also well received. Being a much more complex presentation and an attempt to prove suitable to a wide variety of arthropod-borne virus investigators for utilization in many ways, it was initially designed to be reproduced photographically for two primary reasons. First, it would present the data submitted by investigators exactly as they compiled it; second, change of editions of cards for any particular virus, within range of the information called for under the punch card key, could easily be accomplished.

At the outset it was recognized that the Catalogue would be of changing value according to the information each consultant required. For this reason the format devised contained a great deal of information which might not be of use to a worker in the confines of a laboratory, while those working at distances in the field might find their sole source of information about a number of viruses easily accessible on the catalogue cards and easily located by the punch card system.

Most of the comments on the Catalogue were commendatory and encouraging. A number of suggestions have been received for improvement. As promised with issue of the first pages, the Sub-committee is combining these suggestions into a revised form which will be submitted for review in October. Any suggestions from others not yet heard from will be welcome. Another set of cards for additional strains is now in production and should be distributed within a few weeks.

Finally, it must be remembered that the Sub-committee on Information Exchange has been established with a view to compiling and issuing a newsletter and for production and maintenance of an Arthropod-borne Virus Catalogue. Beyond editorial selection and correction of obvious discrepancies and errors, the Sub-committee is not final judge or jury to pass on technical content which, at times, may be debatable and therefore highly stimulating in the search for facts.

The value of both the newsletter and the Catalogue, therefore, reflect the thought and intensity of interest of the participants. Both have been gratifying to the Sub-committee and with continued participation of similar quality,

these contributions to exchange of significant information among arthropod-borne virus investigators will continue apace.

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REPORT FROM DR. E. RUSSELL ALEXANDER, CHIEF, SURVEILLANCE SECTION,
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Since the last report in this newsletter in April, more data have been accumulated on the occurrence of specific arthropod-borne encephalitis in the United States in 1959. Unfortunately, these reports are still incomplete, but when the remainder are received they will be published in the Encephalitis Surveillance Report of the Communicable Disease Center (PHS). Available reports yield the following summary of human disease in 1959.

There still remains no adequate national reporting category to reflect the true incidence of arthropod-borne encephalitis. These diseases, and many more, are included in a category of "acute infectious encephalitis," of which 2,205 cases were reported in 1959. As usual, this category showed a peak in September, to which proven arthropod-borne encephalitis was a small contributor. Acute infectious encephalitis remains a satisfactory clinical grouping for immediate reporting, and would reflect a sudden increase of any encephalitis before laboratory proof of etiology were available. But for analysis it is less useful. Therefore we have relied on reports of laboratory confirmed cases only. So today, certain arbitrary criteria for classification of cases as confirmed or presumptive must be employed. These definitions are detailed in the Encephalitis Surveillance Report - 1959.

Of the three types of arthropod-borne encephalitis in the United States, Eastern encephalitis (EE) was of most unusual occurrence. The outbreak on the New Jersey coast in the fall was the largest since the Massachusetts epidemic of 1938. In New Jersey, 33 persons were involved, of which 25 were confirmed and 8 were presumptive as due to EE. In Florida, one confirmed and two presumptive cases occurred in Pinellas County in October. One additional presumptive case was reported in Maryland in September.

Saint Louis encephalitis (SLE) was the most prevalent in 1959. One hundred and twenty cases were reported from three foci. In California, 40 cases were confirmed as due to this agent. These occurred from mid-July to late September, with the majority occurring in the latter month. The age distribution of these cases was unusual for recent occurrence of SLE in California, in that there were more cases in the older age groups reported this year. Florida contributed 56 cases, of which 17 were confirmed. Fifty-five cases were from Pinellas County, and reflected the outbreak which occurred in St. Petersburg in October. The epidemiological pattern of this outbreak was similar to other urban outbreaks in the central United States.

Twenty-two cases of SLE were confirmed in Texas, from early July to late October. Prominent in this occurrence were the high plains counties, but no discrete epidemics were discovered. The remaining two isolated cases were confirmed in Missouri and Wisconsin.

Western encephalitis (WE) remained unusual in its relative absence. In California only 23 cases were confirmed. In Colorado one, and in Texas 10.

There is no doubt that more cases occurred in the United States but the relative absence of confirmed cases is highly significant. No outbreaks of this disease came to our attention.

At the time of writing this report, there is no evidence of epidemic occurrence of these diseases in 1960. On the east coast there have been scattered reports of clinical disease in horses, and EE virus isolations have been made from a horse brain in Maryland and from mosquitoes in Florida (Taylor). Further, a case of EE was confirmed in Florida in February, but no cases have been confirmed during this season. Similarly, WE and SLE have not been reported in 1960, except for a few isolated cases in the West. It should be stressed, however, that it is too early to emphasize its relative occurrence.

Once more, contributors to this newsletter will be encouraged to send information on the occurrence of arthropod-borne encephalitis in the United States to the Surveillance Section, Communicable Disease Center, as soon as it is available. Current reports will be issued as soon as such information accumulates.

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

Equine encephalitis is now occurring in Maryland. Evidence is tabulated.

<u>CASE</u>	<u>ANIMAL</u>	<u>COUNTY</u>	<u>APPROXIMATE OCCURRENCE</u>	<u>BASIS OF DIAGNOSIS</u>
1	Pony	Somerset	Aug. 9 - 10	Clinical.
2	Pony	Talbot	Aug. 9	Isolation of virus from brain. Preliminary typing indicates EEE. Examination of brain shows histo-pathological findings compatible with equine encephalitis.
3	Horse	Worcester	Aug. 17	HI on serum from affected animal pos. at 1:320 for EEE.

Total bloods taken, initial and rebleeds	571
Note: The above represents a preliminary calculation.	
Sentinel mice litters exposed (Five litters lost from predatory animals or cannibalism)	138
Sentinel chickens exposed (Two groups of 24 and 26 exposed when one to three days of age at an interval of one month. Bled at four and eight days, one month and six weeks following exposure)	50
Arthropod collections (mostly from light traps) (Including more than 9,000 mosquitoes)	64
Mammals (rodents)	52
Reptiles (seven snakes, one turtle and one tortoise)	9

The use of sentinel mice, which has proved to be so rewarding in the hands of the Causeys in Brazil, for detecting the presence of arthropod-borne virus, was disappointing, as only one isolation was made by this method.

All specimens were examined in chick embryo, duck embryo, hamster kidney and rhesus monkey kidney tissue culture. Virus isolations were made from the blood of five out of 496 birds examined. EEE was isolated from a Purple Grackle, and two viruses from Blue Jays, one from a Grackle and one from a Water Turkey (Anhinga) are unidentified. Examination of mosquito pools for virus is as yet incomplete, but eight isolations have been made thus far. Those viruses identified include EEE from a pool of 118 Culicine mosquitoes and WEE from a pool of nine Aedes taeniorhynchus. The remaining six unidentified isolates from mosquitoes were obtained from three pools of Culiseta melanura, one pool of Mansonia indubitans, and two pools of Culex nigripalpus. As previously stated, only one isolation was made from sentinel mice. This agent is as yet unidentified but resembles, in many respects, the viruses isolated from the Blue Jays. Most of the specimens from sentinel chicks, rodents and reptiles are under examination.

REPORT FROM DR. TELFORD H. WORK, ROCKEFELLER FOUNDATION
VIRUS LABORATORIES, NEW YORK, N. Y.

It was assumed that the Seminole Indians living on reservations in southern Florida, under conditions of continuous exposure to blood-sucking arthropods, should provide sentinel serum specimens which would reflect by HI antibodies the past and current local activity of certain arthropod-borne viruses when tested against a variety of antigens for viruses known from elsewhere in the Western Hemisphere. With the generous assistance of USPHS nurse Mrs. Mable Head, 163 sera were collected from Indians residing in Brighton and Big Cypress Reservations.

Brighton Reservation is an extensive expanse of flat land to the west of Lake Okeechobee. It is largely open grassland with numerous palmetto and hardwood hammocks which provide shade and shelter for clusters of open-sided, thatch-roofed huts in which the Indians live, widely scattered over the reservation. Most of the land is used for communal grazing of cattle. Because there are numerous car tracks and an adequate network of roads, horses are not generally used on the reservation.

Big Cypress Reservation is situated about 50 miles south of Clewiston, the county seat on the southern shore of Lake Okeechobee. This reservation consists primarily of cypress swamp and encompasses a considerable area of everglades. The Seminole residents here speak a different language and are more rural in their pursuits of hunting and fishing and small plot gardening. They move through the country by boat and canoe along myriad waterways of swamp and everglades.

Random selections of sera from both reservations were screened in three dilutions against various combinations of antigens. These included in Group A, EEE, WEE, and Mayaro; Group B, yellow fever, dengue 2, St. Louis encephalitis (SLE), Ilheus, Bussuquara, Powassan, Russian spring-summer encephalitis (RSSE), Murray Valley encephalitis (MVE), and Modoc; Group C, Oriboca, Caraparu and Marituba; Group Bunyamwera, Cache Valley, Guaroa, California, Chittoor and Bunyamwera viruses.

The highest incidence of positives was to Group B antigens with highest titers ranging from 1/20 to 1/160 for St. Louis. There were five positives in children under 15, the youngest being six years old. The over-all positive incidence was 32/163 with 16% positive in Brighton Reservation and 25% in Big Cypress.

There was no evidence of yellow fever or dengue activity in the past, any low titer positive being attributable to overlap from SLE. There was no evidence of Powassan or other tick-borne RSSE complex virus activity.

Nine sera that showed low titer positive reactions to one or another of the Group A (EEE, WEE or Mayaro) antigens were tested simultaneously against all the available Group A viruses including Chikungunya, Semliki, Mayaro, AMM 2021, AMM 2354, EEE, WEE, VEE, Sindbis, Aura, Middelburg and the unidentified strains isolated by Taylor and Henderson in the Lake Placid area. Five showed distinct positive patterns to VEE virus in titers from 1/80 to 1/160 very definitely indicating exposure of these donors to Venezuelan equine encephalitis virus in the past. It is of further interest that the VEE positives were among residents of the Big Cypress swamp; not from Brighton Reservation. In repeat screening of all 163 sera now being done against VEE antigen, there is a high incidence of specific VEE positive patterns in the sera of Big Cypress swamp residents (57% in the Big Cypress Indian population versus only 7% in the Brighton Reservation residents; intermarriage between the two tribes may explain part of the 7% in Brighton).

This is the first substantial evidence of VEE virus activity in North America. The localization in the Big Cypress swampland corresponds to situations

such as the Nariva Swamp in Trinidad where Downs et al. have isolated it, the localities near Belem in Brazil where the Causeys have isolated strains and in Colombia where it has been studied by Sanmartin and Groot.

VEE virus has been transient wherever it has been encountered. Taylor's field attempts to isolate virus this past summer in Florida did not extend to the Big Cypress Reservation so no adequate field attempts have yet been made to recover this virus in this locality.

The sera reacting positively to Bunyamwera group antigen, Chittoor or Bunyamwera, were retested in eight dilutions to Cache Valley, Guaroa, Ilesha, Chittoor, and Bunyamwera viruses. Although the Cache Valley antigen used is not a particularly sensitive antigen, the patterns of positives gave highest titer of 1/20 or 1/40 for Cache Valley virus indicating that this or a closely related virus is the agent responsible for infections of the Seminoles in Florida. Definitive neutralization tests remain to be accomplished.

Of 60 sera tested for Group C antibodies, none were positive indicating that none of these three viruses or close relatives have been active in these localities of Florida.

REPORT FROM DR. J. O. BOND, FLORIDA STATE BOARD OF HEALTH,
ON ENCEPHALITIS STUDIES IN PINELLAS COUNTY, FLORIDA

The purpose of this study is to collect information regarding the presence and activity of arthropod-borne viruses in an area adjacent to the City of St. Petersburg, Florida, where 23 serologically confirmed cases of St. Louis Encephalitis (or closely related Group B virus), and one case of Eastern Equine Encephalomyelitis occurred from August to November, 1959.

In an attempt to isolate an agent or agents and to assess virus activity several survey methods are being employed. These include: (1) collection of bird bloods for isolation attempts and serological study, (2) attempts to isolate viruses from arthropods, and (3) the use of sentinel (suckling) mice.

Using the method of Stamm, Davis and Robbins ("A Method of Studying Wild Bird Populations by Mist-Netting and Banding," Stamm, Donald D., Davis, David E., and Robbins, Chandler, S.) 317 wild birds were captured, banded and bled (from the external jugular) during the period June 1 through June 19, 1960. The species captured included Bluejay (44), Cardinal (71), Carolina Wren (25), Common Grackle (42), Great Crested Flycatcher (17), House Sparrow (11), Mockingbird (4), Red-bellied Woodpecker (2), Red-eyed Towhee (1), Red-winged Blackbird (70), Summer Tanager (2), White-eyed Vireo (5), Tufted Titmouse (1), and Yellow-billed Cuckoo (22). It is hoped that these figures (representing the breeding birds) together with recapture data from later catches will provide us with population estimates.

Satisfactory blood specimens were collected from 244 birds, and are now being processed. Light trap catches of mosquitoes were very low during this period due to prevailing dry weather conditions. On several occasions, the catch ran only 2-6 mosquitoes per trap, per night. No isolations have been made from sentinel mice used during this period.

On July 18, 1960, the second mist-netting and banding operations were begun. The completion of this work has been somewhat delayed due to high water in the study area. Preliminary results show a few species of birds to be present which were not in the area during the first study. Also, the density of fresh water breeding mosquitoes was increased appreciably at the beginning of the second study period.

It is anticipated that one or more additional bird and mosquito collections will be made to complete our picture for this season.

REPORT FROM DR. MICHAEL SIGEL, DIRECTOR, VIRUS LABORATORIES,
THE VARIETY CHILDREN'S RESEARCH FOUNDATION, MIAMI, FLORIDA

Given below is a chart showing a series of patients with antibodies to St. Louis. These patients demonstrated clinical signs and symptoms suggestive of encephalitis. With the exception of patient No. 3607 who was ill in September of 1959, the patients were ill in the fall of 1958. In the last few weeks we have tested a serum from one local patient and have obtained very interesting results. This patient had a high titer in the HI Dengue test, titer 1:160 or 1:320. Her symptoms as described by her physician and by herself were those of Dengue, yet she remembers having had similar symptoms diagnosed as Dengue twenty-odd years ago. Her CF tests for St. Louis gave us a titer of 1:32. We are, therefore, puzzled at the moment as to whether our serologic testing is reflecting a current infection or infection in the past. Additional sera are being collected and neutralization tests will be performed as soon as possible.

Acute and Convalescent Sera
of Suspected Miami Encephalitis Cases Tested
for St. Louis Encephalitis and Dengue 2 Viruses

		<u>SLE</u>		<u>Dengue</u>		
		CF	Neut. index	HI	Neut.	
Landrau 2968	S1	1:4		80 R80		Onset 12/30/58
	S2	1:4		40 R80	316	
Russo 3607	S1	64		160		Onset 9/19/59
	S2	128		160		
	S3	128	100	160	ND	
Barta 2744	S1	< 4		Neg.		Onset 10/5/58
	S2	16	68	Neg.	Neg.	
Belles 2781	S1	< 4				Onset 10/20/58
	S3	16	10	<10	Neg.	
Vanderbeck 2837	S1	< 4		<10		Onset Fall 1958
	S2	16				
	S3	16				
	S4	16	32	<10	Neg.	

R = Repeat

One of the most interesting findings obtained in recent weeks has been the isolation of a virus from a pool of the culicine mosquitoes. This virus has not yet been typed but preliminary tests suggest that this is not Dengue, St. Louis, Eastern or Western virus.

REPORT FROM A. C. PIPKIN, MSC, USN MEDICAL SCIENCE LIAISON UNIT,
THE GORGAS MEMORIAL LABORATORY, ON ARTHROPOD-BORNE VIRUS RESEARCH

Work in virology at the Gorgas Memorial Laboratory dates to the initiation of investigations on the insect vectors of yellow fever in 1949, when the infection progressed, during a 3-year period (1948-51) from the Colombian border to the southeast to the Costa Rican border to the northwest. A second outbreak occurred in 1956-57, but no cases were seen northwest of the Panama Canal, and no further trace of yellow fever has been elicited, either as an immunity in animal hosts, isolation of the virus itself, nor as human infection.

The original work in 1949, in addition to a survey of possible insect vectors, included animal reservoir surveys, and investigation of suspected human cases. As a natural extension of this work, investigation of the other arthropod-borne viruses was commenced. Preliminary surveys have been made since then in many parts of Panama, and a continuing survey has been carried out at Cerro Azul, some 30 miles to the southeast of the capital city.

A 3-year project embracing investigations of the arthropod-borne viruses in general is being carried out in the Bocas del Toro region up along the Costa Rican border of Panama. This area produced a considerable number of cases of yellow fever in man during the 1948-51 outbreak. The project includes the laboratory identification, as far as available facilities permit, of the viruses isolated, their hosts, and etiological relationships. The work is being pursued partly through a cooperative arrangement with the Middle American Research Unit, which is based at the Gorgas Hospital in the Canal Zone.

So far, among the viruses identified, are the St. Louis encephalitis virus, and the Ilheus encephalitis virus from mosquitoes; the St. Louis encephalitis virus from human blood, and the Ilheus encephalitis virus from the blood of the blue heron and from a Toucan, both from the Bocas del Toro area. A survey of human blood for antibodies to Ilheus encephalitis virus in the Darien province, in the southeast region of Panama, would indicate also that this virus produces widespread human infection in the Darien area.

REPORT FROM DR. ALEXIS SHELOKOV, DIRECTOR,
MIDDLE AMERICA RESEARCH UNIT,
BOX 2011, BALBOA HEIGHTS, CANAL ZONE

Recent arrival of Drs. James M. Brennan and Conrad E. Yunker, acarologists assigned to MARU from the Rocky Mountain Laboratory of the National Institutes of Health, marks the initiation of a project on the possible role of mites in transmission of virus diseases. This exploratory investigation will be conducted in collaboration with the staff of the Gorgas Memorial Laboratory in Panama City and the Malaria Control and Survey Branch of the Surgeon's Office, U.S. Army CARIBBEAN.

The long-term collaborative project with the Gorgas Memorial Laboratory (GML) on the ecology of arthropod-borne viruses in Panama is continuing. Five more viral agents have been isolated: two from phlebotomus sandflies and three from mosquitoes.

The earliest phlebotomus isolate (BT-78) has been shown to be serologically related to the almost simultaneous isolate (GML-122) from another pool by Dr. Rodaniche of GML; apparently it is not related to the Sicilian or Naples Sandfly Fever viruses. From two portions of a more recent phlebotomus lot (BT-1256), tested separately in the two laboratories, similar new virus strains were isolated. The MARU strain (unlike the prototype BT-78) was isolated only in suckling mice; on inoculation of HKTC and MKTC with later passage material characteristic CPE was produced; the virus was shown to be related to both the MARU BT-78 prototype and the earlier Dr. Rodaniche's isolate (GML-122). A new low titering SM isolate from phlebotomus (BT-766) resembles the earlier agent (BT-104), which undoubtedly is not related to the BT-78 phlebotomus prototype.

The Psorophora sp. isolate, BT-219, yielded a good CF antigen which has been shown to be unrelated to the two 1958 GML and MARU isolates from Culex dunnii, Phlebotomus BT-78, as well as EEE, SLE and EMC viruses known to be active in Panama. Attempted preparation of HA antigen has not been successful. Three new isolates, BT-740 and BT-1343 from C. vomerifer, and BT-1122 from Anopheles spp., have been propagated in SM.

An exploratory laboratory project was initiated recently to expand observations of others on the field use of filter paper discs in viral diagnostic procedures.

REPORT FROM DR. W. G. DOWNS, DIRECTOR,
TRINIDAD REGIONAL VIRUS LABORATORY, PORT OF SPAIN, TRINIDAD

One of the laboratory technicians suffered a mild infection with a recently (Trinidad) isolated VEE strain. By the end of 31 days after infection, he showed NT changes in keeping with this diagnosis, which is supported by isolation and reisolation of virus from his blood. However, there has been no detectable HI response to this infection. The individual furthermore had no pre-existing Group A immunity.

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

Result of a Serological Survey in Chile and Peru,
by Hemagglutination-Inhibition Test

Forty sera from adult residents of Santiago, Chile, and 171 from residents of different localities in Peru were tested for HI antibodies. Forty-four Peruvian sera were from persons twenty years old or less and the localities of origin were: Lima, Cañete, Ica, Nazca, and Canta. The sera were tested against fifteen antigens, as follows: Group A - EEE, WEE, Venezuelan EE and Mayaro. Group B - St. Louis encephalitis, Bussuquara, Ilheus, dengue type 2 and Russian spring-summer. Group C - Marituba, Oriboca and Caraparu. Bunyamwera Group - Bunyamwera, Guaroa and Cache Valley virus.

Chile. One serum was positive at dilution 1:40 against WEE antigen only; four additional sera had low titer antibodies, 1:20 and 1:40 against several of the group B antigens, SLE, Bussuquara, and Ilheus. These results showed little activity by any of the 15 viruses studied. Furthermore, no conclusion could be reached concerning the etiology of the group B antibodies, owing to the low titers and lack of specificity within the group.

Peru. Group A. Two sera were found positive, one with Mayaro, the other one with Venezuelan EE. Group B. Thirty-eight sera of 140 residents of Lima, Cañete, and Ica were positive; while only one of 31 residents of Nazca and Canta was positive. Twenty-two of the sera gave titers 1:80 or better. There were among the sera three different types of antibody pattern as follows: 1) In at least nine sera, mostly from Lima, the pattern was compatible with infection with SLE virus. 2) In at least six sera, none of which were from Lima, the pattern was compatible with dengue type 2 infection. 3) In at least six sera, the pattern was of a secondary immune response.

In conclusion, evidence of activity of at least two group B viruses - possibly SLE and dengue type 2 - was found in three localities in Peru. Group C. Not a single serum reacted against any of the three viruses. Bunyamwera Group. Twenty-seven sera were positive against Cache Valley antigen; only one positive came from Nazca or Canta. As the titers were consistently low, 1:20 or 1:40, the interpretation is difficult; it would seem, however, that either Guaroa, or another virus of this group related to Guaroa, has been active in three of the surveyed localities.

REPORT FROM DR. J. R. SCHMIDT,
U.S. NAVAL MEDICAL RESEARCH UNIT NO. 3, CAIRO, EGYPT

The Departments of Virology and Medical Zoology of the U.S. Naval Medical Research Unit No. 3 (NAMRU-3) in Cairo, U.A.R., have initiated a study of the role of birds and ticks as vehicles for long distance

dissemination of arbor viruses. The Unit is advantageously located at the base of the Nile delta, where migratory birds converge during their annual migrations between Europe and/or Asia and Africa. Bloods from representative avian species and ticks parasitizing the birds will be processed for virus isolation. It is expected that surveillance of birds in migration will yield realistic information on their importance in introducing "new" viruses into formerly non-endemic areas and in providing a pre-season infective source in regions where viruses appear to be absent in the winter.

REPORT FROM DR. HERBERT C. BARNETT,
WALTER REED ARMY INSTITUTE OF RESEARCH, WASHINGTON, D. C.,
ON SANDFLY FEVER STUDIES

Efforts to isolate viruses from sandflies collected in West Pakistan and Iran have continued. Ninety-three lots containing 11,000 sandflies have been triturated and passed through suckling white mice to date. Twenty-eight viral isolates have been obtained from 77 lots of Phlebotomus females tested, 3 isolates have been obtained from 15 lots of Sergentomyia females tested, and one isolate has been obtained from the only lot of male Phlebotomus tested. Five isolates have been reisolated from the original sandfly inocula but further efforts at reisolation have been suspended, pending grouping and identification of the entire series of 32 isolates. Nine of the isolates have been obtained from material collected in Pakistan and 23 from material collected in Iran.

The isolate from male Phlebotomus (collected in Iran) and the three from female Sergentomyia (collected in Pakistan) are of particular interest. All four viruses are still unidentified but their biological properties suggest that they may belong to the sandfly fever group. Should the isolate from male sandflies belong to this group, further evidence of transovarial transmission of virus in sandflies will have been obtained. Virus isolation efforts for the remainder of the year will be devoted to the testing of males. If the isolates from Sergentomyia are identified as sandfly fever viruses, a new group of sandflies will be under suspicion as vector species.

Identification of the viral isolates is being undertaken by the Section of Infectious Disease, University of Maryland School of Medicine. Identification of isolates has been hampered by low titer in almost all of the isolates. Two isolates recovered from Phlebotomus collected in Pakistan appear to be indistinguishable from the Sicilian strain of sandfly fever by neutralization test. One isolate from Phlebotomus collected in Iran behaves biologically like a sandfly fever virus but does not appear to be related either to the Sicilian strain or to the Naples strain of sandfly fever by neutralization test. Efforts are now being made to develop hemagglutinating antigens and to group the isolates by neutralization screening tests. Efforts are also under way to isolate viruses from acute phase sera collected in Pakistan from febrile patients.

REPORT FROM DR. H. S. HURLBUT,
U.S. NAVAL MEDICAL RESEARCH UNIT NO. 2, TAIPEI, TAIWAN,
Box 14, APO 63, San Francisco, Calif.

Information on arthropod-borne viruses

The results of the investigations of Japanese encephalitis on Taiwan which were carried out by Dr. J. Thomas Grayston and his co-workers during 1958-1959 have been submitted for publication. The findings include the first isolation of JE virus on Taiwan and serological evidence of the distribution of this agent in man and animals. The virus isolations were from mosquitoes. Culex tritaeniorhynchus is considered the important vector. In addition to 22 isolations made from C. tritaeniorhynchus, one was obtained from Culex fuscocephalus.

Dr. Grayston has returned to the United States. Work on JE and other arthropod-borne viruses is being continued by Dr. H. S. Hurlbut. Mosquitoes are being received for virus isolation from Taiwan and Okinawa.

During the present season in the month of July, 87 cases of encephalitis attributed to JE virus infection were admitted to the Hospital of the National Taiwan University Medical School. According to Dr. T. C. Kao, Director of the Hospital, the number of cases has been unusually large. There were 30 deaths.

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

Work has continued on field specimens collected in the Philippines in 1956 and in Thailand in 1958, and in addition, on Japanese B vaccine. Certain elements of progress are indicated below.

I. Philippine Hemorrhagic Fever

Since finding that most patients with the "H" fever syndrome in Thailand had more or less concomitant infections with chikungunya and a dengue virus, it was deemed wise to extend the serological studies on the Philippine patients to include a number of group A viruses. HAI with representative group A viruses failed to indicate involvement of the A group in Philippine "H" fever.

II. Mosquito isolates from the Philippines

P-886 from Culex bitaeniorhynchus was readily reisolated. It has been compared further with Sindbis, to which it was shown earlier to be closely related. By both HAI and neutralization in our hands it appears to be a

distinct agent and probably should not be considered a strain of Sindbis. It was given to Dr. Casals for his special comparison of Sindbis related strains.

P-422 from Aedes aegypti, now in excess of 60 suckling mouse passages continues to have an incubation period of 3 days, a titer of only about 10^{-3} or 10^{-4} , affects no other laboratory animal tested and produces no CPE in any of a very large number of cell lines employed and fails to persist through serial passage in many of these. It continues to defy grouping or other identification. It has been given to Dr. Casals.

P-759 (C. quinquefasciatus), P-830 (C. gelidus), P-870 (Mansonia uniformis), and P-935 (C. fuscocephalus) have all shortened their incubation period through serial suckling mouse passage to 3 days and are in the process of identification.

P-581 from Culex fuscocephalus, after over 30 suckling mouse passages has a titer of about $10^{-6.5}$, kills only suckling mice but produces CPE in MKTC and HKTC. It makes a good CF but not HA antigen, does not appear to belong to groups A, B, C or several of the smaller groups and has been turned over to Dr. Casals for further identification.

P-301 from Aedes aegypti and identified as dengue type 3 has been successfully transmitted in the laboratory by Aedes aegypti, the first experimental transmission of this new virus of the dengue group.

III. Thai Hemorrhagic Fever

Serology and virus isolation and identification still support the conclusion that several dengue viruses predominantly dengue 2-like, and a chikungunya-like agent were etiologically involved in 1958. Many persons were infected with dengue and the group A agent almost simultaneously. Why these agents produced a hemorrhagic fever syndrome in Bangkok and immunologically closely related or identical agents produce a dengue-like syndrome elsewhere is still unknown.

Further immunologic study of the TH-SMAN virus, from a patient, originally tentatively typed as dengue 1 has indicated that though closely related to type 1 it also appears to have antigenic components of several other types to such a degree that it is substantially different from Hawaiian type 1. Paired patient sera, from the first of which the isolation was made, show a very significant antibody rise to the homologous virus.

Several of the virus isolates from patients are still poorly adapted and do not behave like most members of the group (dengue 2 and chikungunya). Some of these have now been lost in passage. As a whole these patients represent mild, atypical disease and probably should not have been called "H" fever on a clinical basis.

Continued comparisons of TH-35, the prototype chikungunya-like virus, with chikungunya and N'yong nyong and other closely related agents

show TH-35 to be closest to chikungunya but not necessarily identical. So far, however, we still consider it a probably minor strain difference only. Our work confirms that of Porterfield who sent us N'yong nyong that it can be differentiated from chikungunya but is more closely related to it than to Semliki Forest, Mayaro and others in the subgroup.

IV. Mosquito isolates from Thailand

T-1, T-4 and T-93 from Aedes aegypti, like viruses from patients (TH-36 prototype), are closely related to dengue type 2. T-55 from the same mosquito species has been partially identified as similar to dengue 2.

T-96 also from Aedes aegypti is a suckling mouse agent apparently not belonging to groups A or B. During later passage it appears to have become contaminated with a virus with many characteristics of LCM and it titers higher now in adult than in suckling mice. This has delayed progress.

T-185 from C. quinquefasciatus has been identified as a member of group A and is closely related to chikungunya and Semliki Forest. We anticipate that since chikungunya was prevalent this may be the virus, but in a Culex rather than in an Aedes as expected.

T-172 and T-174 appear to have been isolated from Culex quinquefasciatus and on preliminary identification are EEE. Since a strain of EEE from New Jersey had been handled in the laboratory 2 days before the mosquitoes were ground up, contamination was suspected. However, after over a month with no EEE or related work done in the whole department, reisolation was accomplished readily from T-172 but not from T-174 frozen mosquito suspension. If contamination occurred it had to be during the preparation of the original mosquito suspension of T-172 and from this possibly a further contamination of T-174. Comparisons of characteristics of the N. J. strain and these two possible new isolates is under way and presently certain differences appear to exist. Homologous neutralization is better than heterologous and one virus titers higher in HKTC than in suckling mice and the other behaves in the opposite manner. No conclusions as to the validity of these isolates can be drawn as yet, but we are beginning to have less hesitation in considering them bona fide. HAI tests on 10 available Bangkok horse sera, though revealing group A antibody, do not suggest EEE as the source of that antibody.

V. Japanese B Vaccine Studies

Efforts to produce or select an attenuated mutant by laboratory manipulation continue and some ground has been gained. Some success in producing plaques has finally been accomplished. Loss of virulence by the i.c. route has not been conspicuous (2 to 3 logs decrease only) but loss of virulence by a peripheral route in suckling mice is far more encouraging and possibly more significant.

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

Arthropod-borne Virus Disease in California, 1959-1960

During 1959 there was little evidence of activity of Western equine virus in California. There were two cases of encephalitis where the diagnosis was presumed to be Western equine encephalitis, based on a high titer of CF antibodies for this virus in the convalescent serum specimen, but a rise in titer of CF antibodies was not related to the disease and therefore a definite diagnosis cannot be made. There were two nonfatal cases of horse encephalitis which were proved to be caused by Western equine virus, one by demonstration of a rise in titer of virus neutralizing substance and the other by a rise in titer of CF antibodies for this virus in tests of acute and convalescent blood serum specimens. There were 41 cases of St. Louis encephalitis in 1959. There was no grouping of cases to suggest a focus of infection but nearly all of the cases were in the northern half of the Central Valley. Only one of the patients died of the disease. There was one case of Colorado tick fever but the tick exposure occurred in Nevada. Blue tongue of sheep was recorded in 17 flocks of sheep in 10 counties in 1959. The seasonal occurrence was April to November.

Mosquitoes were collected in Kern County in 1959 and tested in Dr. W. C. Reeves' laboratory at the University of California, School of Public Health. The California State Department of Public Health conducted a mosquito surveillance study in Fresno County and these mosquitoes were tested in the Viral and Rickettsial Disease Laboratory. All of the mosquitoes were negative for Western equine virus. St. Louis virus was isolated from Culex tarsalis mosquitoes collected in both Kern and Fresno Counties but the virus did not appear in mosquitoes until August.

In 1960 there has been only one case of Western equine encephalitis in man. The date of onset was May 15th and investigation revealed that the infection was contracted in the Imperial Valley of Southern California. There have been no proved cases in horses in 1960 and only a few cases of horse encephalitis were reported during the first six months of 1960. Two cases of St. Louis encephalitis have been identified, both with onset in mid-July. The California State Department of Public Health did not continue the mosquito surveillance study in 1960. Dr. Reeves has not reported any isolations of virus from mosquitoes collected in Kern County. There have been seven cases of Colorado tick fever. One of these was exposed by tick bite in Wyoming. There were three with onset in April, one in May, and three in June. The diagnosis was made by isolation of the virus from the blood in one case, otherwise the diagnosis was made by demonstration of a rise in CF antibodies for Colorado tick fever virus in tests of acute and convalescent blood serum specimens.

Field Studies in 1960

There have been four different field projects in 1960. One was an ecological survey in Mexico carried out in cooperation with other agencies. A summary report is attached. One virus was isolated in Mexico. This was a strain of Rio Bravo virus obtained from a pool of salivary glands of five Mexican freetail bats, Tadarida mexicana, collected in Hermosillo, Sonora, April 1, 1960, in a mist net. The virus was reisolated and identified by the serum-virus neutralization test. The serological studies of human, horse, chicken and rodent sera were done in Dr. W. C. Reeves' laboratory. The studies already completed show evidence of past infection with Western equine virus and St. Louis virus in horses and St. Louis virus in man.

Two field expeditions were made to the Great Basin Plateau in 1960. The first was from May 23 to June 1 and the second from July 8 to 13. A total of 70 small mammals were bled for virus studies. Blood specimens from a pocket mouse, Perognathus parvus and a chipmunk, Eutamias amoenus, trapped near the Big Sage reservoir on May 27, and a golden mantled ground squirrel, Citellus lateralis, trapped at Hackamore, May 31, were positive for Colorado tick fever virus. Both of these study areas are in the Modoc National Forest, Modoc County. These strains of virus were identified by cross immunity test. Several strains of virus have been isolated from Dermacentor andersoni ticks collected in Modoc County in 1960. These appear to be Colorado tick fever virus but the cross immunity tests have not been completed. The degree of tick infestation in small mammals can be judged by the observation that one Peromyscus maniculatus wood mouse carried one Dermacentor nymph and 119 Dermacentor larvae. A serological survey of horses from the Great Basin Plateau showed that of 78 horses, 12 had antibodies to St. Louis virus and 25 to Western equine virus. Two of the horses with antibody to Western equine virus had been vaccinated. There is a history of sporadic cases of horse encephalitis in the Great Basin Plateau area since 1927. Most of the positive horse bloods came from ranches in the higher mountain valleys at an elevation of >5,000 feet. Cases of human plague occurred in the Warner Mountains of Modoc County in 1948 and 1950. There was an epidemic of plague in ground squirrels and chipmunks in Modoc County in 1936-1937.

A study of mosquitoes was conducted at a horse farm near Sacramento where there was a proved case of Western equine encephalitis in a horse with onset on April 1, 1959. All mosquitoes that could be found in March were tested for virus. Collections were made each week by the staff of the Vector Control Bureau in Sacramento. These were tested in 42 pools and were all negative for virus. There were 93 Anopheles freeborni, 2 Anopheles punctipennis, 81 Culiseta inornata, 2 Culiseta incidens, 6 Aedes increpitus, 3 Aedes sierrensis, 53 Culex tarsalis, 16 Culex pipiens, 25 Culex erythrothorax, and 8 Culex peus.

A study of nestling birds was conducted in the Sacramento River Basin of the Central Valley. There were 6 colonies of tricolor redwinged blackbirds exceeding 100,000 adult birds. Nestlings were collected each week during the nesting season June 8 to July 13. The tricolor redwinged blackbirds were about one month later in nesting than in 1959. They do nest

later than the Brewer blackbirds and the bicolor redwinged blackbirds which had completed their first nesting in May in the river valleys. A total of 161 tricolor redwinged blackbird nestlings were bled and tested for virus. One virus was isolated from a tricolor redwinged blackbird collected on July 13. This virus has not been identified as yet. This was one of the last three nestlings found. A total of 45 nestling and immature birds of other species were tested in 1960 and they were all negative for virus. The one tricolor blackbird colony is in a large marshland, and in the fall the migratory bicolor blackbirds and Brewer blackbirds in this marsh usually exceed 5,000,000 birds. This study site will be used for collection of mosquitoes and immature birds in September.

Laboratory Studies

There are three major fields of interest in the laboratory studies. One is the identification and characterization of newly isolated viruses. The second is the study of relationships of the three group B viruses isolated in California, and third, the studies of virus strains of Western equine virus and St. Louis virus in an effort to obtain variants that may prove suitable for use as live virus vaccines.

A new virus has been isolated from the spleen tissue of a Yuma myotis bat, Myotis yumanensis, collected June 9, 1956, at the Borel Power Station, Kern Canyon, Kern County, California. This was one of 15 pregnant bats collected at that time. Most of these were tested right away but a few were left until February, 1960, when we completed the remaining bat specimens stored from previous years. The specimens had been stored in a sealed glass tube on dry ice. Twelve of 18 infant mice sickened or died within 11 to 18 days following inoculation with the suspension of the spleen tissue. Re-isolation was accomplished with some of this suspension stored until July, 1960. In the re-isolation, 10 of 18 mice sickened or died and on subpassage all of the mice were dead or prostrate in four days. At the time of the re-isolation 6 adult mice were inoculated with the original suspension. One was prostrate on the 23rd day. The new virus, which will be called the Kern Canyon virus, is distinct from the viruses previously isolated in California. Mice immunized IM with the Kern Canyon virus are immune to intracerebral challenge with the homologous virus but not to Western equine, St. Louis, Colorado tick fever, Modoc, Rio Bravo, or California viruses. The virus has the same characteristics as the California and Cache Valley viruses as regards the incubation period and disease picture in infant mice inoculated IC and IP and in adult mice inoculated IC. It is not pathogenic for adult mice when inoculated IM.

The cross immunity studies of Rio Bravo, Modoc, and Powassan viruses show that Modoc virus given by IM inoculation in mice will produce immunity to both Modoc and Rio Bravo viruses. Immunization with the Rio Bravo virus by IM inoculation produces solid immunity to the homologous virus but not to Modoc virus. There is no relationship between Modoc and Powassan viruses by the cross immunity test. There is no relationship between Modoc and St. Louis virus by the cross immunity test but mice immunized with the Powassan virus

show considerable resistance to St. Louis virus. The remarkable thing in the cross-neutralization tests is that the serum of hamsters which were inoculated with a single dose of Modoc virus neutralizes 4 to 5 logs of the homologous virus but less than 1 log of the Rio Bravo virus, yet immunization with the Modoc virus produces solid immunity to IC challenge with the Rio Bravo virus. It is thus obvious that it is necessary to do cross immunity studies before one can characterize a new virus with certainty. Another interesting example of partial cross immunity was obtained when mice were immunized with a tissue culture strain of Western equine virus that had been isolated from a naturally infected bird by tissue culture in hamster kidney cells. These mice were subsequently shown to be solidly immune to the homologous virus given IC. They were held an additional month and then challenged with Eastern equine virus IC. There was a high degree of resistance with survival of more than 50 per cent of the immunized mice. Controls of the same age and sex all sickened and died.

The various passage studies of St. Louis virus in chick embryos has resulted in the development of one strain which ordinarily does not kill adult mice inoculated intracerebrally. Only an occasional mouse sickens and dies following inoculation with 10^{-1} to 10^{-7} dilutions of the tissue virus suspension. This virus has been maintained in chick embryos by constant cultivation in the yolk sac since isolation from mosquitoes. The yolk sac is used as a source of virus and the inoculation is by the yolk sac route. The virus maintained for the same time in yolk sac passage using the chick embryo as a source of virus did not lose its pathogenicity for adult mice. Another line of St. Louis virus derived from mosquitoes has been maintained in chicks by IM passage. After 30 passages this line shows an increased pathogenicity for adult mice as indicated by a gradual shortening of the incubation period from 8 to 5 days. Serial passage of Western equine virus for >100 passages in chick embryos has not reduced the pathogenicity of this virus for adult mice. In fact, one line now kills 30 per cent of the adult mice inoculated IM. A line of Western equine virus maintained in chicks by IM passage showed no reduction in pathogenicity for adult mice after 50 passages. The titer of the virus in the blood increased from 10^{-3} to 10^{-8} . The use of the plaque technique in the search for avirulent strains of Western equine and St. Louis viruses is under investigation.

Field Study of Arthropod-borne Viruses
Hermosillo, Sonora, Mexico
March 26 - April 9, 1960

A field study and demonstration of techniques for a general ecological survey for arthropod-borne viruses was conducted at Hermosillo as a part of the program for the Eighteenth Annual Meeting of the United States - Mexico Border Public Health Association. The field work was completed during the week of March 26 - April 2 prior to the regular meeting which was held in Hermosillo April 4-8. The Third Plenary Session was devoted to a program of papers on arthropod-borne viruses and this was followed by a barbecue lunch at Jardin Corona, Hermosillo, where an exhibit was arranged illustrating the field study program. The various types of field equipment were on display,

such as mammal traps, insect traps and collecting equipment and a mist net for collecting birds and bats. Live specimens of the common small mammals and skins of the rarer species were exhibited on a row of tables with the common and scientific names listed on cards. A display board was set up for exhibiting photographs of the various aspects of the field work, and these were mounted around a chart listing the animal and arthropod specimens collected during the field study.

Objectives of Study

The principal objective of the field study and demonstration was to develop a good working relationship between the scientists of Mexico and the United States having a mutual interest in the study and control of arthropod-borne virus diseases in the border region. The field demonstration included a population study of arthropods, small mammals and birds of the Hermosillo region. Mosquitoes were collected in the city of Hermosillo and from ranches nearby for virus studies. Ticks were collected from cattle for virus studies. Fleas were collected from small mammals for identification only. Breast tissue specimens were taken for virus studies from all of the small mammals which were pregnant or lactating when trapped. Blood serum specimens were collected from man, horses, chickens and the larger of the wild rodents for a serological survey for arthropod-borne viruses. The arthropod and tissue specimens were stored in sealed glass tubes on dry ice. Two pools of Culex tarsalis mosquitoes, a pool of salivary glands from Tadarida mexicana bats and two mammal breast tissue specimens were tested for virus at the field laboratory by intracerebral inoculation into infant mice. Some of the mosquito specimens were set aside for testing in the Virus Laboratory in Mexico by Dr. Carlos Campillo Sainz and Dr. Julio de Mucha Macias. The rest of the mosquito specimens will be tested by Dr. W. C. Reeves at the School of Public Health, Berkeley. The tick specimens will be tested at the Viral and Rickettsial Disease Laboratory, California State Department of Public Health, by Dr. E. H. Lennette, Director of the Laboratory. The mammal tissue specimens will be tested by Dr. Harald N. Johnson of the Viral and Rickettsial Disease Laboratory, Arthropod-Borne Virus Studies Project. The serological studies of the serum specimens will be done at the School of Public Health by Dr. W. C. Reeves. The identification of the wild mammal specimens was done by Mr. Murray. The identification of fleas and ticks will be done by Dr. Vargas.

REPORT FROM DR. WILLIAM C. REEVES, PROFESSOR OF EPIDEMIOLOGY,
UNIVERSITY OF CALIFORNIA SCHOOL OF PUBLIC HEALTH,
BERKELEY, CALIFORNIA

This report reviews accomplishments during the period March 16, 1959, to April 1, 1960, in studies on "The Ecology and Control of Arthropod-borne Viruses" in Kern County, California.

Biological and morphological studies of the overwintering population of female Culex tarsalis indicated that it is unusual for this species to take a prewinter blood meal. This would appear to limit the possibility of this vector serving as an overwintering virus reservoir.

Western equine encephalitis (WEE) virus was isolated from a naturally infected white-crowned sparrow in midwinter. This is a significant finding as it indicates virus persistence in a naturally infected host in the absence of continuous vector transmission. In addition, confirmation was obtained of the persistence of WEE for one month and longer in the organs of experimentally infected wild birds. Similar persistence has not been shown for St. Louis encephalitis (SLE) virus.

A project has been established to determine the effect of intensive C. tarsalis control on the transmission rates of WEE and SLE viruses.

Conditions in the summer of 1959 were unusually unfavorable to endemic maintenance of WEE and SLE virus. An acute shortage of irrigation water and resultant low vector population were identified as major factors limiting virus activity.

Species-specific precipitin antisera have been developed by use of antigenic fractions of avian sera to sensitize chickens or by the development of immunological tolerance to crossing antigens in chickens being sensitized with whole serum. These antisera will be useful for identification of the sources of mosquito blood meals.

Tissue culture techniques developed for virus study have included: neutralization tests for serological studies, study of WEE and SLE plaque characteristics, and utilization of tissue culture systems for virus isolations from field samples.

Serological surveys of residents of Kern County indicated that HAI and CF antibodies for WEE and SLE were more transitory than expected. Rodent sera had a high prevalence of SLE antibodies and few had WEE antibodies. A short-term field study in Hermosillo, Mexico, revealed that WEE and SLE viruses had been active in that area.

Studies of two myxomatosis outbreaks in California led to virus isolations from Anopheles freeborni and Sylvilagus bachmani.

REPORT FROM DR. ARCHIE D. HESS, CHIEF,
ENCEPHALITIS SECTION, TECHNOLOGY BRANCH,
U.S.P.H.S., COMMUNICABLE DISEASE CENTER, GREELEY, COLORADO

Study of the natural history of the encephalitides has continued at the Greeley, Colorado; Bakersfield, California; Wenatchee, Washington; and Taunton, Massachusetts, Stations. Supplementary observations were made in six additional sites in the western states during 1959.

Western and St. Louis Encephalitis

Transmission rates for WE and SLE in 1959 were obtained from sixty chicken sentinel flocks bled in October at 8 locations in the western states, and from farm flocks from 2 additional areas. Hemagglutination-inhibition (HAI) antibody rates in the sera of these chickens provided the index of WE and SLE transmission rates at each site. These data, when added to similar data collected in the past 3 to 5 years and to the history of WE and SLE epidemics, provide evidence of definite regional patterns of virus activity. In the zone north of the 75° isotherm, transmission rates of WE have been high (35 to 65%), but SLE rates have been consistently low (less than 10%). South of the 75° isotherm both WE and SLE are active, although one of the viruses may be significantly more active than the other in any given year. In 1956 and 1959 SLE rates predominated over WE in the Greeley area, whereas in 1955, 1957, and 1958 WE antibody rates were higher than SLE. Virus isolations from mosquito pools substantiated the serologic results. Comparison of various climatic factors with transmission rates in the study areas is currently in progress.

An intensive comparative study of 3 ecotypes was made during the 1959 season. The study areas are described as follows: (1) a bottom-land area adjacent to a small river, containing marshes and sloughs and diverse ground cover varying from dense to sparse; (2) an upper sonoran dryland prairie having short grass cover and an intermittent stream bordered by deciduous trees; (3) an isolated farm containing sloughs associated with a relatively constant-flow spring-fed stream surrounded by dryland prairie. The mosquito, bird, and mammal populations were studied both qualitatively and quantitatively, and evidence of virus activity was sought in the fauna of each area, and by means of sentinel chicken flocks.

Results of the studies indicate that the river area and the intermittent stream area have comparable virus transmission rates which are significantly higher than the rates in the isolated farm site. Magpies, pigeons, and other bird species were present in the areas of high transmission, but absent from the area of low transmission. Peromyscus and Sciurus are abundant in the areas of high transmission, but scarce or not present in the area of low transmission. Although the river area and the intermittent stream area had comparable virus transmission rates, the former area had a much larger mosquito population. A study of the ratio of parous to nulliparous mosquitoes from each area is under way.

Certain small mammals are currently under investigation as possible overwintering hosts and sources of the infection from which the bird-mosquito cycle might be initiated. The ground squirrel (Citellus tridecemlineatus) following intradermal inoculation of 300 LD₅₀ of WE virus, had a viremia. Culex tarsalis became infected when feeding upon infected Citellus, and subsequently transmitted the infection. Field specimens of Citellus, Mus, Perognathus, Lepus, and Peromyscus have had HAI antibody, suggesting some contact with WE and SLE viruses or closely related viruses. Of 89 specimens of Peromyscus collected in early spring of 1960 in the river area study site, 40 had antibody to WE, and 17 of 113 had antibody to SLE.

Pursuant to the search for factors which limit encephalitis virus activity, a serologic survey was made in the adjacent mountain region near Greeley where ecologic conditions are known to differ from those found in the Greeley study areas. Seventy-seven per cent of domestic chickens sampled had antibody to WE virus but only 2 per cent had SLE antibody. WE antibody was found at an altitude of 8,000 feet. A study of the movement of Culex tarsalis up the mountain canyons and the nature of the virus transmission in the high areas is currently in progress.

Eastern Encephalitis

Comparative studies in eastern encephalitis virus transmission were made at 4 sites in the vicinity of the Taunton, Massachusetts, field station. One site was located in a Culiseta melanura-breeding swamp; the other 3 sites were located 0.1, 0.5, and 2.0 miles from the swamp. A chicken sentinel flock was located at each site, and the chickens were bled at intervals during the summer of 1959. The results indicate that the transmission rate in the swamp site was about twice as high (65%) as the rates for the other 3 sites (35%). Furthermore, antibody was detected first in the swamp site flock, and subsequently in the other flocks. These data appear to substantiate the theory that swamps producing Culiseta melanura serve as enzootic foci of infection. The considerable virus activity in chicken flocks located near human habitations and the lack of recognized human or equine cases during 1959 in the Taunton, Massachusetts, area substantiated the theory that virus activity may occur in the wild bird populations without necessarily involving man or domestic animals.

Vector-host Relationships

Studies on the influence of visual factors in the orientation of blood-seeking Culex tarsalis, using standard rotary tests, have shown that significantly more mosquitoes are captured by a solid black trap than by a trap with a 1-inch checkerboard pattern. This latter trap captured significantly more than a trap with a 2-inch checkerboard or a transparent trap. Thus it appears that orientation of blood-seeking mosquitoes is influenced by visual as well as chemical stimuli.

Host preference studies of the primary vector of EE virus, Culiseta melanura, indicated that this mosquito had the highest attraction and attack rate on birds, but was also attracted to and fed upon mammals and snakes.

This is particularly significant with regard to the potential of C. melanura as an epidemic vector, since it had previously been assumed that it rarely feeds upon mammals.

New Jersey Encephalitis Outbreak

Investigation of the 1959 outbreak of encephalitis was made in co-operation with the New Jersey State Health Department. EE virus was isolated from a pool of non-engorged Culex restuans, from a sparrow brain, a pheasant brain, and 3 horse brains. One-third of 100 wild bird bloods collected in the outbreak area, and 65 per cent of 203 chicken bloods had HAI antibody to EE virus. At least one of the wild birds had HAI and neutralizing antibody to both EE and WE virus.

Experimental Control of Encephalitis

During FY 1960 detailed background information for experimental control of SLE and WE was obtained in three study areas: (1) Kern County, California; (2) the Quincy area of the Columbia Basin, Washington; and (3) the High Plains area of Texas. Data obtained from these areas include population indices and encephalitis infection rates for mosquitoes and wild birds and mammals, SLE and WE transmission rates in avian sentinel flocks, and infection rates in resident human populations.

Each of the three western study areas shows a different pattern of transmission. In Kern County transmission rates are high for both WE and SLE and C. tarsalis is believed to be the primary enzootic and endemic vector for both viruses. In the Quincy area transmission of WE is very high but SLE is low, and the sole vector is presumably C. tarsalis. In the High Plains area transmission rates are high for both SLE and WE, and both rural SLE (C. tarsalis-transmitted) and urban SLE (C. quinquefasciatus-transmitted) appear to be present. HAI antibody tests on human sera collected from this area at the end of the 1959 season indicate that human infection is primarily due to SLE even though the avian sentinels show high antibody rates for both SLE and WE. This suggests that the human population may be exposed mostly to C. quinquefasciatus and control operations will have to be directed against this species if transmission to humans is to be prevented.

REPORT FROM DR. CARL M. EKLUND,
U.S.P.H.S. ROCKY MOUNTAIN LABORATORY, HAMILTON, MONTANA

Over 20 garter snakes which were inoculated with western equine virus either on September 14-28 or November 6 were circulating virus soon after they came out of hibernation in late March, April and May. The longest period that virus was detected after coming out of hibernation was 57 days. The snakes which were brought into the laboratory after emerging from hibernation and kept at room temperature had the shortest period of viremia, 2-3 weeks. Those snakes that were kept outside where the nights were cold had

a much longer period of viremia. Although virus was detected in some snakes as soon as they came out of hibernation, in other snakes it took a few days before they started to circulate virus. Over 90 per cent of the snakes, that came out of hibernation, circulated virus. We were unable to carry any Culex tarsalis through the winter.

To date 286 snakes collected in nature have been bled and no virus has been isolated. Blood and tissues from 677 wild birds collected either during the spring or fall months when there were no mosquitoes have been examined for the presence of virus. No western or St. Louis virus has been isolated. Approximately 3000 Dermacentor andersoni from Colorado have been examined this spring and early summer in an attempt to get another isolation of the Powassan-like virus. This attempt was unsuccessful. At the present time blood is being collected from small mammals for attempted virus isolation and antibody surveys.

During the past two years, in cooperation with Dr. Olson of the Public Health School at the University of Minnesota, blood from sentinel pigeons in various areas in Minnesota has been studied. It is of interest that in a southern part of the state no western antibodies were acquired by any sentinel pigeon while in the northwestern part of the state, in the Red River Valley, 70 per cent acquired antibodies during 1958 and 30 per cent in 1959. There apparently can be a complete absence of virus in one section of the state while there is a high incidence of infection in another, although there are C. tarsalis and birds in both areas.

This summer the western equine virus has been isolated from C. tarsalis in both eastern Washington and western Idaho. It appears that this will be a year with a high incidence of western infection.

REPORT FROM DR. W. L. POND, ARTHROPOD-BORNE VIRUS SECTION,
LABORATORY OF TROPICAL VIROLOGY, NATIONAL INSTITUTE OF
ALLERGY AND INFECTIOUS DISEASES, NATIONAL INSTITUTES OF
HEALTH, BETHESDA, MARYLAND

The activities of the Arthropod-borne Virus Section, National Institutes of Health, Bethesda, Maryland, for the period December, 1957, through December, 1959, have been described in their Technical Report No. 1 which was recently distributed to interested persons. A brief indication of the contents of this technical report may be of interest.

1. Reactivity of sera from human residents of Guatemala, the Republic of Panama, and Mexico to certain arthropod-borne viruses. Studies have been carried out on sera from residents of these countries in order to determine the presence or absence of various types of antibodies to certain arthropod-borne viruses. These results are in a preliminary state and generalities cannot be made at this time.

2. A study of the occurrence and importance of arthropod-borne viral diseases in tropical and sub-tropical areas of the United States.

Similar studies have been made also of the sera from residents of the Miami area and also sera have been collected for this purpose from residents in Texas. A few attempts have been made to isolate agents from individuals ill with possible arthropod-borne viral infections and also tests are under way on acute and convalescent phase sera from these persons. It is of interest that Dr. Joel Ehrenkranz, in his work in the greater Miami area, has observed aseptic meningitis occurring seasonally in epidemic form. Aseptic meningitis, possibly of arthropod-borne virus origin, appears to be an important cause of disease of man in that area.

3. Investigations of the comparative rates of reaction of arthropod-borne viruses with homologous and heterologous antibodies. Studies of this Section indicate that by altering the time allowed for incubation of virus-serum mixtures, the specificity of the reaction can be enhanced. If the virus-serum mixtures, to be used in a neutralization test, are not incubated prior to inoculation, it appears that only homologous antibody reacts and the reaction of heterologous antibody will be absent or at a minimum.

4. Development of a practical and specific flocculation test for the demonstration of arthropod-borne virus antibodies. Attempts are being made to apply the "Bentonite" flocculation test to the detection of arthropod-borne viral antibodies. The system seems to be workable with the arthropod-borne viruses tried up to now. However, many difficulties have to be overcome before the test is applicable for general field use.

5. Evaluation of available tissue culture systems as practical tools for propagating, quantitating, and isolating arthropod-borne viruses. As a part of a long range project, 10 arthropod-borne viruses have been tested in various tissue culture systems in order to obtain some idea as to efficacy of the particular tissue culture system in sustaining growth and in evidencing the presence of the particular viruses.

6. Investigation of the susceptibility of "germfree" mice to arthropod-borne viruses and testing of "germfree" mouse colony for presence of latent viruses.

7. Use of "germfree" mouse tissues in tissue culture for propagation and quantitation of arthropod-borne viruses. It appears that dengue type 1 virus can be grown to a moderately high titer and also shows cytopathogenicity to the same titer when grown in tissue culture prepared from germfree mouse kidneys.

8. Demonstration and characterization of a newly observed hemonuclear adsorption reaction which indicates the presence of virus in infected tissue culture systems. Not only does the phenomenon of hemadsorption occur with some viruses but also a subsequent phenomena has been observed in which the cytoplasm of chick erythrocytes has been stripped leaving the nucleus intact and adsorbed onto tissue culture cell sheets.

9. Attempts to grow arthropod tissues in vitro. Many and varied attempts have been made to grow arthropod tissues successfully in vitro. No success has as yet been obtained except with silkworm ovarian tissue as reported by others in the literature. When this later tissue was inoculated with certain of our arthropod-borne viruses, no growth or CPE was observed.

10. Pathogenesis of Ilheus virus in adult and suckling mice. Adult and suckling mice were infected by Ilheus virus in this laboratory and subsequent levels of viremia were determined and correlated with the age and the route of inoculation used.

11. Transmission studies on Ilheus virus using mice as hosts and *Aedes aegypti* mosquitoes as vectors. It has been possible to transmit Ilheus from mice to other mice by means of the *Aedes aegypti* mosquito as a vector. Studies were made on the extrinsic incubation period and on the relationship of the transmissibility of the virus as related to levels of virus present in the blood of the donor animals.

12. Longevity studies on laboratory reared mosquitoes which are to be used in investigations on virus-host-environment interrelationships.

Investigations indicated above are being continued in the Arthropod-borne Virus Section at the National Institutes of Health and progress in these and in other additional investigations now under way will be reported in subsequent newsletters.

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

Work with the ARBO viruses in this laboratory is now represented by the tissue culture studies of Dr. Ned H. Wiebenga.

The investigations have centered around the study and characterization of several sub-strains of dengue 1 virus that have been adapted to different cell culture systems, including a carrier culture in human skin cells adapted to horse serum. After 15 months' continuous propagation (and up to 2 years) the virus in carrier culture fluids differed from the mouse-adapted prototype in that it consistently produced CPE in association with infection of human skin cells adapted to horse serum (HuS 2806 cells) and of monkey kidney cell cultures. Using CPE as an indicator of infectivity, tissue culture harvests titered in the range of 10^4 to 10^7 infective doses. Serological identity has been confirmed by neutralization of mouse infectivity and tissue culture CPE as well as by fluorescent antibody staining by specific antiserum prepared in rabbits with the mouse-adapted virus.

Another sub-strain obtained by rapid serial passage through human skin cells adapted to human serum (HuS 2544) behaved differently than either the mouse-adapted or HuS 2806 cell-adapted virus. While CPE was produced

within 72 hours after infection with dilutions as great as 10^{-9} or 10^{-10} , infectivity for suckling mice was lost completely after the 3rd serial passage in cells and could not be re-established. Technical difficulties have thus far prevented serological identification of the cytopathogenic agent either by serum neutralization of CPE or by fluorescent antibody staining. Current efforts to demonstrate a hemagglutinin for chick or goose cells in the tissue culture harvests have been unsuccessful to date.

NOTES FROM DR. WILLIAM F. SCHERER,
UNIVERSITY OF MINNESOTA MEDICAL SCHOOL, MINNEAPOLIS,
ON THE SEITZ EK FILTER AND ITS INSIGNIFICANCE

Initial inquiry

April 28, 1960

Republic Seitz Filter Corporation
17 Stone Street
Newark 4, New Jersey

Dear Sirs:

We have used extensively the small Seitz EK filter pad for Swinney syringe adapters supplied by Becton-Dickinson Company. Upon writing BD Company I was informed that the pore size for Seitz EK filters is 0.1 micron. Could you please inform me whether this is the minimum pore size or the average pore size? ... Also, could you let me know...how you measure the pore size of a filter.

I shall appreciate your prompt reply.

Sincerely yours,

Rob Ra, M.D.

First Reply

May 2, 1960

Brochure arrives with letter stating:

"The pore size of our filter pads was determined by passing known particles of standard sizes through the pads to determine which were retained and which were passed.

We trust this information will serve you."

Very truly yours,

Republic Seitz Filter Corp.

Second inquiry

June 7, 1960

Republic Seitz Filter Corporation
17 Stone Street
Newark 4, New Jersey

Dear Sir:

Thank you for your letter of 2 May 1960 and accompanying brochure which describes your Seitz filter pads for Swinney adapters. I still have some further questions, however, which I would appreciate your answering.

1. "What do you mean by pore size range?" You list the "pore size range" of an EK filter as 0.1 micron. How can a single figure be a range? Is this an average pore size or a minimum pore size?
2. What kind of particles of known standard size do you use to determine pore size of your filter?
3. When you speak of pore size, do you mean the size of the hole in the filter or do you mean the size of the particle that goes through the filter?
4. How do you calculate the size of the hole in the filter from the known size particle which passes through it?
5. What diluent do you use for the particles of known size which are used for standardization of filters?

We shall greatly appreciate your prompt reply.

Sincerely yours,

Rob Ra, M.D.

Second Reply

June 16, 1960

Dr. Rob Ra
The Medical School

Dear Dr. Rob Ra:

We are replying to your letter of June 7 concerning filter pads for Swinney adapters. In answer to your inquiries, we wish to submit the following information.

1. By "pore size range" we mean to convey the information that each porosity of pad will hold back particles as large and larger than the dimension shown on the literature.

2. The particles used for the determination of the capacity of filter sheets in the clarifying grade were known sizes of charcoal. For the "GP" and "S" grade we used Serratia marcesens. The last three grades EK, S1 and S3 were evaluated by our parent company, the Seitz Company of Germany, and I do not have a record of the exact particles used by them for their determinations.*

3. We believe our explanation No. 1 will answer this.

4. We do not calculate the hole size, we simply grade the pad according to the particle size which it retains. In actual operation asbestos filter pads will retain, by adsorption, particles of a smaller size than the actual interstices of the pad so that size of hole is not necessarily the determining factor.

5. Distilled water was used to hold the suspended particles in all tests.

We trust the above information will help you.

Very truly yours,

Republic Seitz Filter Corp.

Third inquiry

June 30, 1960

Republic Seitz Filter Corporation
17 Stone Street
Newark 4, New Jersey

Dear Sir:

Thank you for your letter of 16 June 1960 which answers most of our questions.

However, we are still a bit confused about the procedure for evaluating "pore size range" for EK filters. Would it be possible for you to find out from your parent company, the Seitz Company of Germany, what kind of particle is used for this determination?

Also, could you tell us whether you spot check the "pore size range" by periodically sending some of your filters to Germany? Or was this determination done merely a number of years ago and you only follow the procedure for making the filters originally worked out by the Seitz Company.

Moreover, since you do not have a record of the exact particles used for the EK filters, can I infer that you do have a record that distilled water was used to suspend the particles used to test EK filters?

*Underlining by Editor.

I am sorry to keep bothering you, but this information is extremely important to us in our research.

Yours truly,

Rob Ra, M.D.

Third Reply

Dr. Rob Ra
The Medical School

Gentlemen:

We are replying to your letter of June 30 with further reference to pore size range determination.

We are sorry we gave you the wrong impression about our relationship with the Seitz Company of Germany. While it is true that they were our parent company, we were forced to part company with them during World War II. Since then, like many children who have left their parents, we are no longer on speaking terms.

You can therefore see that it is impossible for us to send to Germany for any information, so that you are correct when you state this determination was done years ago and we have been following the procedure laid down by the Seitz company. We can, however, assure you that all determinations were made with distilled water.

I would now like to ask a question concerning your research. As we are in the process of setting up new evaluating procedures, could you recommend standard uniform sizes that we could use for this purpose? We would certainly appreciate any recommendations you could offer us.

Yours truly,

Republic Seitz Filter Company

Editor's Note

The above exchange of correspondence is factual. Any resemblance to filters of known pore size is purely coincidental.

NOTICE TO MEDICAL AND VETERINARY MICROBIOLOGISTS ABOUT
THE SERUM EXCHANGE LIST PREPARED AT THE DEPARTMENT OF
VETERINARY SCIENCE, UNIVERSITY OF WISCONSIN,
FOR THE WILDLIFE DISEASE ASSOCIATION

THE PURPOSE OF THE SERUM EXCHANGE LIST

The serum exchange list is prepared by a committee of the Wildlife Disease Association. The list consists of names and addresses of individuals or institutions that are interested in furthering research on diseases of wild animals. Cooperation among the participants may be in one or more of three categories.

1. Collecting and making available serums of wild animals.
2. Providing facilities for receiving and storing serums.
3. Performing serological tests on serums made available.

Cooperators may participate to the extent that they choose. The collection of serums will often be part of an organized field program. Deer serums are obtained in Wisconsin by biologists at check stations maintained during the deer season. In most instances, serological testing will be part of a going program. In Wisconsin a variety of avian and mammalian serums are tested for antibodies to Eastern Equine Encephalitis on a program supported by National Institutes of Health.

THE USE OF PAPER DISCS IN THE COLLECTION OF WHOLE BLOOD AND SERUM FROM WILDLIFE

Paper Discs

An absorbent paper disc 17 mm in diameter manufactured by Carl Schleicher and Schuell Company of Keene, New Hampshire, is used to take blood samples. Each disc adsorbs 0.2 ml of blood or serum.

Blood from Living Animals

Paper disc adsorbed samples can be obtained from living animals by saturating discs by application to an incised vessel, such as a wing vein in birds or ear or tail veins in mammals. These small incisions require no after care. In taking blood from larger animals where adequate quantities can be drawn by venipuncture, the use of paper discs has some practical application since the dried blood or serum samples require no refrigeration during transportation and storage.

Blood from Dead Animals

Recent experience with the collection of paper disc adsorbed whole blood samples indicates that samples suitable for virus neutralization tests can be obtained from birds and mammals some time after death, even after carcasses have been frozen and thawed. This permits the utilization of animals killed along roadsides and birds killed by collision with wires, towers,

etc. Blood is collected from dead animals by merely incising the thorax and the heart, lungs and large blood vessels. Fluid blood or serum which has separated from coagulated blood is removed by saturating paper discs.

Mailing and Storage

Discs are air-dried on any convenient nonabsorbent surface, after which they may be mailed to the laboratory in a double envelope. Refrigeration is not required. Long storage at room temperature appears not to affect the quality of adsorbed antibodies. Recovering blood proteins by elution in 0.6 ml volumes of distilled water or broth results in a dilution of about 1/4, a factor compatible with most serological procedures.

Application

In studies on eastern equine encephalomyelitis in Georgia, air-dried blood and serum samples from horses and wild birds were collected on discs of white absorbent paper 17 mm in diameter. Six-tenth ml eluates prepared from these discs were tested for neutralizing antibodies against eastern equine encephalomyelitis virus by inoculation of virus-disc eluate mixtures into chicken embryos. Results were compared with those derived from tests on the fluid serums. Further evaluation of the technique consisted of a comparison of replicate tests on dried and fluid serum samples collected during the course of hyperimmunization of a rabbit. The results of serum neutralization tests conducted with paper disc eluates compared favorably with parallel tests on fluid serum samples. Complement fixation tests were also successfully conducted with eluates of paper disc adsorbed samples of rabbit serum. It is concluded that paper discs may be recommended for use in epizootiological studies where blood samples are to be collected from small animals and birds, thereby facilitating the handling of small volumes and obviating the need for refrigeration during their collection, shipment and storage.

Reference:

Karstad, Lars, J. Spalatin, and R. P. Hanson (U. Wisconsin, Madison), 1959. Application of the paper disc technique to the collection of whole blood and serum samples in studies on eastern equine encephalomyelitis. J. Inf. Dis. 101: 295-299.

Editor's Note:

Newsletter Number 23 of the Wildlife Disease Association, issued in January, 1960, contains a report by the Serum Exchange Committee listing a large variety of serum collections available in North America. The editor is Dr. Robert Holdenried, Fort Detrick, Frederick, Maryland.

LIST OF ANNUAL AND OTHER REPORTS RECEIVED

Annual Progress Report (March 1959 through April 30, 1960)

Arthropod-borne Virus Research Unit
Division of Epidemiology
School of Public Health
University of California
Berkeley 4, California

Annual Report (16 February 1959 to 15 February 1960)

Research on Attenuated Living Vaccines for Arthropod-borne Virus Diseases
School of Medicine
University of Maryland
Baltimore 1, Maryland

Technical Report Number 1 (December 1957 - December 1959)

Interim Technical Report on the Major Research Activities of the Arthropod-borne Virus Section
Laboratory of Tropical Virology
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland

Report on Studies of Arthropod-borne Virus Infection in Jamaica, West Indies, 1959

Department of Pathology
University College of the West Indies

Annual Report for 1959

Trinidad Regional Virus Laboratory
Port of Spain, Trinidad, WI

Viral Encephalitis in Florida (June 1960)

Division of Epidemiology
Florida State Board of Health
Jacksonville, Florida

California Surveillance Reports on Viral Diseases of the Central Nervous System, 1 and 2
(through August 13, 1960)

CNS Surveillance Unit
Bureau of Acute Communicable Diseases
State Department of Public Health
2151 Berkeley Way
Berkeley 4, California

Annual Report of the Gorgas Memorial Laboratory for 1959

Gorgas Memorial Laboratory
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This list is not closed. It is open to requests and suggestions for addition of others professionally engaged in or responsible for arthropod-borne virus investigations.