ECOLOGICAL INVESTIGATIONS PR
ARBOVIRAL DISEASE SECTION
BOX
, COLORADO 80

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number Three April 1961

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INTRODUCTORY NOTES FROM THE SUB-COMMITTEE ON INFORMATION EXCHANGE

MEETING OF WHO STUDY GROUP ON ARTHROPOD-BORNE VIRUSES

By invitation of the WHO, a "Study Group on Arthropod-borne Viruses" met in Geneva, Switzerland, September 5-10, 1960. This group was comprised of nine members, two consultants, four members of the WHO secretariat and one observer. The members of the group represented or rather were engaged in work on arthropod-borne viruses in eight different countries, namely Colombia, Czechoslovakia, India, New Zealand, Uganda, United Kingdom, United States, and USSR.

The meeting was called "to assist in implementing" recommendations for a cooperative international program of investigation of the arthropod-borne viruses proposed at an informal meeting in Lisbon in September 1958 and approved and formalized by a WHO Scientific Group on Virus Research which met two months later in Geneva.

The preliminary report of this Study Group has now been issued and the final report will soon be printed and ready for distribution. This report with annexes covers more than 60 pages and since it may be obtained from the WHO only the main subjects considered are given here: 1) Procedures for determining the presence, prevalence and importance of arthropod-borne viruses in unexplored or incompletely studied areas; 2) Grouping and classification; 3) Importance of arthropod-borne viruses in human and/or veterinary disease; 4) Ecological aspects of the problem; 5) Reference laboratories; 6) Current and potential information exchange procedures; 7) Control measures; and 8) Implementation of the recommendations of the Study Group.

It will be recalled that following the recommendations of the WHO Scientific Group on Virus Research which met in November 1958, the WHO was not in a position to implement these recommendations and an American Group, sometimes referred to as the Gould House Group, was assembled by the Rockefeller Foundation to consider activating some of these recommendations among American investigators with the hope that the experience thus gained might be useful in eventual internationalization under the auspices of the WHO. It is gratifying to report that the activities of this American group, particularly as concerned the establishment of a reference laboratory, efforts to investigate methods of preparation of diagnostic reagents for distribution, and the activities of the sub-committee on Exchange of Information, involving the issuing of a newsletter and the assembling of a virus registry or catalog were presented and approved by this Study Group.

It was recommended, for example, that in addition to a central reference laboratory, regional laboratories be established and a system of collaboration and exchange of material be developed; and that the newsletter as well as the catalog be internationalized. However, as the WHO is not in a position at this time to assume responsibility for either the newsletter or the catalog it was

advised that for the present both remain in the hands of the American group and that international collaboration and distribution be arranged directly or through foreign correspondents.

The integration of the activities of the American group into an international program under the auspices of or in collaboration with WHO will be discussed at the next meeting of the American group when a WHO representative will be present. This meeting is to take place during the second week in April.

The final paragraphs of the Study Group report on the "implementation of the recommendations" are of sufficient interest to reproduce:

"It is recognized that in order to conduct progressive development and implementation of the recommendations of this Study Group, a continuous means of consultation must be maintained. The development of this programme should be planned and financially supported on a long-term basis and should provide for the active participation of recognized experts in this field acting as consultants and as members of an advisory group specially devoted to problems of arthropod-borne diseases. Meetings of research workers should be arranged periodically to review progress achieved, exchange information and propose promising lines of activities.

"This can best be done by an organization such as WHO which would ensure the international co-ordination necessary for steady progress in the type of programme recommended in this report."

Richard M. Taylor, M.D., Chairman

THIRD ISSUE OF THE NEWSLETTER

Beyond acceptance of the newsletter as a valuable means for exchange of scientific information by the WHO Study Group on Arthropod-borne Viruses and the recommendation that it be expanded to international participation, the present issue shows increased interest and value by the participants themselves. There is a larger number of contributions ranging from those which report amazing achievements to some of permanent worth as reference material, unavailable from any other single source. The contents also reflect careful thought and consideration of space by being brief summaries of the essence of considerable accomplishments.

It is the largest issue so far and exceeds in size our original anticipation. If and when the newsletter is internationalized, it appears necessary to consider more than two issues a year. This means additional work which is willingly accepted because it reflects success of an effort to solve the real problem of rapid exchange of specialized scientific information on a global basis. It also reflects the vitality of research effort in this field which is an appreciation of its challenges, importance and opportunity for future scientific achievement.

Telford H. Work, M.D., Editor

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

The work of the Rockefeller Foundation Virus Laboratories in New York is an integral part of the Rockefeller Foundation's virus program. In New York, the main effort is on the study of the basic properties of the arbor viruses. Many of these studies are oriented towards the development of methods which might be of value to the workers in the field.

A summary of some of the results covered in the 1960 annual report follow.

Biophysical and biochemical studies

1. Further studies on the properties of Semliki Forest virus particles. The sedimentation constant of the virus particles was accurately determined with the aid of an analytical ultracentrifuge equipped with ultraviolet absorption optics to be 345 Svedberg. The previous report that no HA smaller than the virus particles exists in a brain suspension of three-day-old mice at a concentration higher than 2 per cent has been confirmed by the results from leading-boundary sedimentation studies. The HA and infectivity are simultaneously inactivated in cell culture media at 37° C at apparently the same rate. On the other hand, the latter, but not the former, is inactivated by a prolonged dialysis at 3° C. These inactivations probably do not involve the destruction of viral RNA.

2. Growth of SFV in tissue-culture cells. HeLa cells give higher titers of both intracellular and extracellular virus than human amnion cells and also manifest stronger cytopathogenic effect. The virus populations produced from these different cells are indistinguishable as far as the ratio of IC titer to IP titer in adult mice is concerned.

3. Modification of nucleic acid metabolism in HeLa cells induced by SFV infection. The following events occur during the "latent" phase of SFV growth curve: (1) The amount of cellular RNA first decreases and then increases. (2) This change is approximately paralleled by the rate of syntheses of DNA and nuclear RNA. (3) The material decrease of total RNA is accompanied by a comparable decrease of P^{32} content in the RNA. (4) The P^{32} content of purine nucleotides becomes strikingly weak compared with pyrimidine nucleotides. (5) A minor nucleotide appears. (6) Soluble RNA is polydisperse.

Tissue culture studies

The main efforts during the year have been the study of the effects of the growth medium with HeLa (Gey) cells on the propagation of different

arbor viruses. It has been found that a rich growth medium is essential for the demonstration of CPE with those agents such as the various strains of dengue and Argentinian hemorrhagic fever which have a long incubation period in tissue culture. By the use of the rich growth medium, the control uninoculated cells maintain their normal morphology for several weeks before showing signs of degeneration. The growth medium which has been found satisfactory consists of 30 per cent human serum, 10 per cent foetal bovine serum, 15 per cent tryptose phosphate broth and 45 per cent Hanks' solution. It has also been found that the adjustment of the medium of the pH to 7.2 to be of great importance.

Virus classification

Group immune response. In the immunological identification of an arbor virus, it has been found profitable to place it first in one of the antigenic groups. Extensive studies on the response of an immune animal when inoculated with another member of the same group are reported. Studies of such a nature with viruses belonging to Group A and Group B are discussed. As a result of these studies, it has been found possible to prepare group immune sera in experimental animals which react in the HI test with all the members of the group used for immunization purposes. This immunological overlap was more marked in HI tests than in CF or neutralization tests.

Studies on strain differences. In the course of studies on the arbor viruses in these laboratories, evidence has been accumulated that there exist demonstrable, immunological differences between different strains of what are considered to be one virus.

An extensive study of the antigenic relationship between the various members of the tick-borne complex of viruses is drawing to a close. The methods of study included antibody absorption and agar gel precipitation techniques. The total number of strains studied was twenty-five. Eleven strains of Central European viruses from seven different countries have been compared. Omsk hemorrhagic fever was represented by four different strains, KFD by three and Far Eastern RSSE by four. Only one strain each of louping ill and Langat virus were included in the study. As a result of these studies, six antigenically distinct viruses have been recognized. In all instances, different strains of the same virus have reacted in an identical manner when examined by both techniques regardless of the date of isolation, the original source, or the passage history. The geographic distribution of these viruses is as follows:

Louping ill	British Isles
Central European	Central Europe including western USSR
Omsk hemorrhagic fever	Central USSR
Kyasanur Forest Disease	India
Forest Eastern RSSE	Eastern USSR
Langat	Malaya

It seems apparent that each viral entity exists in a region which appears, at this time, to be discontinuous from the others. This idea of a distinct regional distribution of the various viruses would seem to have rather important implication in our understanding of the natural history of this viral complex.

Sindbis. Strain differences between different isolates of what appears to be one virus are reported from studies with Sindbis. The strains used in this investigation were 1) AR 339 isolated by R. M. Taylor in Egypt, 2) SA AR 86 isolated by Kokernot and Smithburn in South Africa, 3) IA 1036 isolated in India by Johnson, Kerr, and Shah, 4) AMM 2215 isolated by Buescher in Malaya, 5) P 886 isolated in the Philippines by Hammon and Rudnick. In these studies several HI techniques were employed. Antisera to the various strains were tested by the standard HI test (serum dilutions and constant amount of antigen); by the mixture dilution method and finally by titrating the amount of antigen in a given dilution in the presence of a constant amount of the immune sera. These studies indicated that the two African strains fall into one class while those of India, Malaya and the Philippines are in another. There is a marked tendency of the antisera prepared from the African strains to inhibit agglutination equally well with antigens prepared from all the strains whereas the sera prepared from the Asiatic strains give higher HI titers with homologous antigens than they do with heterologous antigens prepared from African strains.

Similar studies on strain differences are at present in progress with Chikungunya, Mayaro, EEE and Semliki forest virus.

Virus classification by serological means has been one of the main functions of the New York laboratories. During 1960, extensive studies were undertaken on the following virus strains:

Group A. AMM 2215 from Malaya and MRM 39 from Australia. Both clearly belong to the Sindbis complex. Onyong-nyong has been shown to be a new group A agent related to Chikungunya.

Group B. AMM 1775 from Malaya, SAAR 1776 from South Africa and the virus of turkey meningo-encephalitis, from Israel, appear to be three distinct new group B agents. A virus, Co Ar 41922, from Colombia has been shown to be a strain of Bussuquara but can be distinguished from the prototype by antibody absorption methods.

Bunyamwera group. Strain AMM 2222 from Malaya has been shown to be indistinguishable from the Chittoor virus isolated in India.

Ungrouped. AMM 2325, isolated in Malaya, has been found to be related to AMM 2549, another Malayan agent. The relationship, however, could only be shown by cross CF tests. Extensive immunological studies failed to show any relationship between these Malayan viruses and other arbor viruses.

Serum antibody survey techniques

Considerable space of the report is devoted to the techniques of serum antibody surveys by the use of hemagglutination inhibition tests. An attempt has been made to summarize the experience obtained since the introduction of HI tests as a survey tool. The importance of the very distinctive HI antibody pattern following primary infection with each virus is emphasized. The chief difficulties encountered in the interpretation of both HI and NT results is largely due to our lack of knowledge of the norms to be expected following infections with the various arbor viruses.

In a discussion of the NT, the extraordinary diverse results that can be obtained with a given immune serum depending on the age of the mice, the route of inoculation, the strain and the nature of the virus preparation and the presence of the labile factor is emphasized.

Some results of recent HI surveys with sera from residents of Guatemala, Peru, and the Seminole Indians of Florida are given.

REPORT FROM DR. ROBERT L. KAISER, SURVEILLANCE SECTION, U.S.P.H.S., COMMUNICABLE DISEASE CENTER, ATLANTA, GEORGIA

Preliminary data for the year 1960 reveals a total of 1,841 cases of "acute infectious encephalitis" reported in the United States. This category, as has been previously stated, includes infectious encephalitis of several etiologies with proved arthropod-borne encephalitis usually representing a fraction of the total. Compared with the 1959 total of "acute infectious encephalitis," the 1960 total is down about 25 per cent.

The year 1960 was characterized by a sparsity of human arthropod-borne encephalitis. No human outbreaks of the disease were reported in 1960. Preliminary data indicates the following distribution of arthropod-borne encephalitis during 1960 in the United States.

Eastern encephalitis which was prominent in 1959 occurred only in isolated cases. One non-fatal case was noted in Florida during February. Several equine cases of eastern encephalitis were reported from Maryland, Virginia, Georgia, and Florida.

Only 12 documented cases of St. Louis encephalitis in California have come to our attention. Texas reported two.

A total of 16 cases of western encephalitis were diagnosed in Texas and one in California. A small outbreak of western encephalitis in horses occurred in Wyoming and a few cases were noted in New Mexico and Arizona.

Although incomplete, the data tend to indicate a uniformly limited activity of the arthropod-borne encephalitides in the United States during 1960. It is also interesting to note the monthly distribution curve of the 1,841 cases of "acute infectious encephalitis" reported in 1960 referred to previously. No summer-fall peak in the curve was evident in contrast to earlier years such as 1956 and 1958. In these years, and others when arthropod-borne encephalitis was present in epidemic form, a pronounced peak in reported cases of "acute infectious encephalitis" occurred during late summer and fall probably reflecting undocumented cases of arthropod-borne encephalitis. The relatively low incidence of infectious encephalitis during the summer and fall of 1960 appears consistent with the few scattered cases of documented arthropod-borne encephalitis observed.

REPORT FROM DR. RICHARD O. HAYES, CHIEF, TAUNTON FIELD STATION, ENCEPHALITIS SECTION, TECHNOLOGY BRANCH, U.S.P.H.S., COMMUNICABLE DISEASE CENTER TAUNTON, MASSACHUSETTS

(The activities at the Taunton Field Station are a cooperative endeavor of the Encephalitis Section, Communicable Disease Center, U.S.P.H.S, and the Division of Communicable Diseases, Massachusetts Department of Public Health)

Neither human nor equine cases of EE have been detected in the Commonwealth since the 1956 outbreak, but either the virus or the specific antibody have been revealed to be present in Massachusetts each year from 1957 to the present.

The reduction of mosquito breeding sites due to a lack of rain resulted in relatively small mosquito populations in southeastern Massachusetts after July 1960. In fact, discontinuance of several field experiments being conducted in swamp study sites, which usually support large mosquito populations during the summer, was necessitated by the lack of mosquitoes.

A study on the overwintering of Culiseta melanura larvae in two fresh-water swamps was conducted during 1959-60. It was observed that the larvae avoided freezing by entering the mud, and that they burrowed deeper into it as the winter became colder. The lowest mud temperature recorded was 34.5° F., and the larvae were recovered from mud depths greater than 6 inches. Living larvae were collected throughout the period of the study; no collections were attempted in April, but the March collections were made on the last day of the month and the May collections on the second.

In April, May, and June, 1960, a search for eastern encephalitis virus among migrating birds was conducted. Blood samples from 892 birds were collected during their northward journey. All the blood specimens were tested for virus and were found to be negative. Wild birds were collected, banded, bled, and released at two study sites throughout the breeding season. The birds were collected in Japanese mist nets bi-weekly at each of the two study sites from May 10 through September 22nd. A total of 2,400 net hours was spent at each site; 200 and 282 birds were captured at each site, respectively. From 82 of these birds, a total of 189 repeat blood specimens were obtained. Five blood samples was the maximum number of repeat specimens obtained from any given bird (identity made possible by the use of bird bands), and this number was obtained from two different birds. Conversion from negative to positive for eastern EE antibody was detected only in a single bird.

REPORT FROM DRS. ROBERT J. BYRNE,
EDWARD L. BUESCHER, AND GORDON M. CLARK

DEPARTMENT OF VETERINARY SCIENCE, UNIVERSITY OF MARYLAND
DEPARTMENTS OF VIRUS DISEASES AND ENTOMOLOGY, WALTER REED ARMY INSTITUTE OF RESEARCH
WILDLIFE RESEARCH LABORATORY, PATUXENT WILDLIFE RESEARCH CENTER

Equine encephalitis is a late summer-early fall disease which has affected equines in tidewater Maryland since recorded history of the region. Repeated implication of Eastern equine encephalitis (EEE) virus

as the etiologic agent of the disease since 1933, has focused the attention of practicing veterinarians and horse owners upon the disease. In recent years reporting of the disease has been rather complete with 45 cases known to have occurred in 1955, 75 in 1956, 1 in 1958, and 18 in 1959. For the most part EEE has occurred in the Eastern Shore counties of the state, especially in those counties south of the Chester River. Sporadic cases have been infrequently observed on the western shore of the Chesapeake Bay in the five counties which lie between it and the Potomac River (see map, Fig. 1). However no disease had been reported from this area (Southern Maryland) since 1955.

In 1960, at least 16 horses and ponies died from EEE in Maryland (Fig. 1), and one non-fatal infection was observed. Thirteen of the 17 cases were confirmed either by histopathologic examination of the brain and/or virus isolation from the brain, by serologic means (hemagglutination-inhibition and neutralization tests) or by a combination of these methods. The first reported case occurred on August 9 and the last on October 13. Of the 17 cases observed, 14 occurred between August 16 and September 15. It is of further interest that there were 10 cases in Southern Maryland, an area apparently spared since 1955.

In the 1960 season, equine disease also occurred on the Eastern Shore of Virginia, just below the Maryland state line. On October 10, 1960, we were invited to investigate an outbreak of equine encephalitis on Chincoteague Island, Accomack County, Virginia. At that time 14 deaths had occurred in the two preceding weeks. By the time of the last death on October 25, at least 30 ponies had succumbed, 20 on Chincoteague and approximately 10 on the neighboring island of Assateague. Laboratory studies were done on three moribund animals. EEE virus was isolated from the brains of the three studied, and their sera were shown to contain EEE antibody.

On October 13, 14, and 15, mosquitoes were collected in the immediate vicinity of the Chincoteague Island outbreak. A total of 826 mosquitoes: 249 Aedes sollicitans, 29 Aedes vexans, 197 Culex salinarius, 240 Anophles crucians, and 111 Anophles quadrimaculatus, collected while biting horses and humans, from resting stations (sheds and barns) and in light traps, were negative for virus when pools of these mosquitoes were inoculated into day-old chicks and suckling mice.

In early summer of 1960 as part of an initial inquiry into the ecology of EEE in the area, collection of wild birds for virologic and serologic study was extended from the Laurel-Beltsville area, northeast of Washington, to selected sites on the Eastern Shore of Maryland in an effort to sample

areas from which EEE had been reported in the past. Between June and October, plasma specimens were obtained from 308 birds (47 species) resident in this region. Passeriforms were collected by mist netting, on different occasions at 3 separate areas: Blackwater Wildlife Refuge, Cambridge, Maryland; Girdletree Wildlife Refuge, Girdletree, Maryland; and Chincoteague Island, Virginia. Plasma was also obtained from 42 nestling ardieds at Mill's Island, Maryland.

Plasma specimens from 102 of these birds were inoculated into hamster kidney tissue culture but no virus was recovered. Each of the 308 specimens was tested for hemagglutination-inhibiting and neutralizing antibody against EEE virus. A qualitative neutralization test was used which had previously been standardized using avian sera with known neutralization indices. Birds having a positive neutralization test, or an equivocal neutralization test plus an HI titer of 1:10 or greater were considered "positive". Antibody was found in the following species: cardinal (6 positive of 39 tested), wood thrush (1 of 1), catbird (2 of 2), brown thrasher (1 of 3), red-winged blackbird (1 of 20), towhee (1 of 1), tufted titmouse (1 of 12), yellow-throated vireo (1 of 1), field sparrow (1 of 17). The overall prevalence of positive reactors was 4.5%. Highest prevalence, 9%, was found on the Girdletree Refuge in September. No antibody was found in the nestling ardieds at Mill's Island and, surprisingly, no antibody or virus was found in the 58 specimens collected at Chincoteague during the epizootic among the ponies.

It should be noted that all of the positive plasmas were collected prior to the peak of the fall migration. Although failure to find evidence of infection among the birds at Chincoteague may reflect insufficient sampling (no rails and few other shore birds common to the area were tested), it was more likely the result of shifting bird populations caused by the migration.

The positive findings of this preliminary study indicate that EEE virus was widely disseminated over the Eastern Shore in 1960. By combining the facilities and personnel of several interested and collaborating agencies, the feasibility of investigating the ecology of EEE in the area has been demonstrated and future inter-agency collaboration may well clarify the complex problem of the ecology of EEE virus in this region.

TABLE I

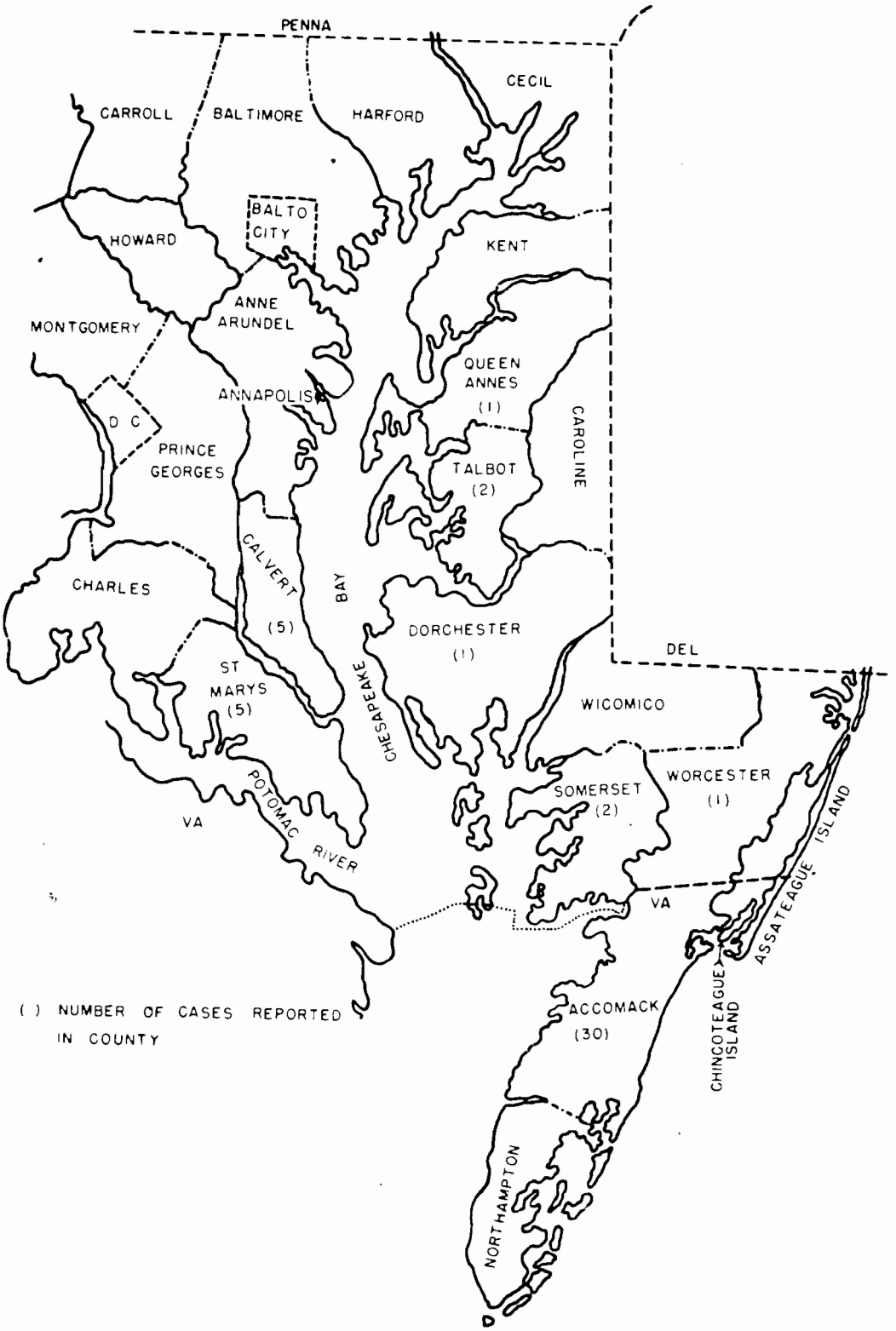
Results of Serologic Tests on Bird Plasma

Area of Collection	Month	Number Tested	Positive neutralization test (1)	Equivocal neutralization test; HI > 1:10 (2)	Per cent Positive
Blackwater	June & July	64	4	0	6
Girdletree	August	48	2	0	4
Mill's Island	August	42	0	0	
Girdletree	September	86	5	3	9
Chincoteague	October	58	0	0	
Total		298	11	3	

1) Intracerebral neutralization test in mice. 1:5 dilution of plasma vs. 75-125 LD_{50} of EEE virus. Test considered positive if less than 2 of 6 mice died.

2) Neutralization test considered equivocal if 3 or 4 of 6 mice died. HI tests utilized 8 units of EEE antigen.

Distribution of EE in Horses and Ponies in the Chesapeake Bay Region in 1960



REPORTS FROM THE VIRUS AND RICKETTSIA SECTION U.S.P.H.S., COMMUNICABLE DISEASE CENTER ATLANTA, GEORGIA

REPORT FROM DR. R. W. CHAMBERLAIN CHIEF, VIRUS-VECTOR UNIT

Summary of field isolations of arbor viruses from mosquitoes in New Jersey, Alabama, and Florida, 1960.

A cooperative study on the South River Game Farm, near May's Landing, New Jersey, was set up in cooperation with the New Jersey State Department of Veterinary Public Health, from May through October, 1960. Another such cooperative project was initiated in the Sawgrass Lake area, Pinellas County, near St. Petersburg, Florida, with the Florida State Board of Health, with collections made from June through October, 1960. A field study of our own was carried out from November, 1959, through December, 1960, on Baptizing Branch, Baldwin County, South Alabama (about 15 miles northeast of Mobile). In each study mosquitoes were trapped periodically and wild birds netted, bled, banded, and released. The results in birds are summarized by Dr. Stamm.

New Jersey

A total of 19, 660 mosquitoes in 1633 pools were tested in 1/2-day-old chicks. Ten virus isolations were made, 4 of EE and 6 of WE. All were from Culiseta melanura except one of EE from Culex restuans and one of WE from an engorged C. territans. The C. territans isolation is of some interest since so far as is known, this species feeds mainly upon frogs.

Table 1 illustrates the seasonal distribution of the isolations. None of the mosquito pools have yet been checked in baby mice for viruses not revealed in the baby chicks.

Table 1. Seasonal distribution of virus isolations from mosquitoes, SRGF, New Jersey, sampled May-October, 1960

	July			August			September			October		
Mosquito Species	EE	WE	ratio infected	EE	WE	ratio infected	EE	WE	ratio infected	EE	WE	ratio infected
C. melanura		1	1/1970		2	2/2059	3	2	5/2874			0/289
C. territans			0/119		1	1/80			0/121			0/72
C. restuans			0/7			0/26	1		1/53			0/33
19 other species			0/2196			0/2444			0/2824			0/261
Total EE							4					
Total WE		1			3			2				

Florida

In the Florida project, 11,000 mosquitoes in 530 pools were tested in 1/2-day-old chicks with negative results. Thus far only a very small proportion of the pools have been inoculated into infant mice. One of these, a pool of C. nigripalpus collected in July, appears to contain virus which brings down the mice on the 3rd day. It has neither been identified nor the possibility of a latent mouse infection ruled out. However, it is presumably not EE or WE or it would have brought down the baby chicks.

South Alabama

A total of 27,326 mosquitoes in 1804 pools captured during monthly collections from November, 1959, through December, 1960, have been tested in 1/2-day-old chicks. Thirteen isolations of EE and 11 isolations of WE have been made, with the seasonal distribution shown in Table 2.

Thus far only a fourth of the pools have been inoculated into baby mice. From these, however, mainly from Anopheles crucians, at least 9 isolations have been obtained, which bring down baby mice in 2-5 days. These have not yet been identified nor the possibility of latent infections in the mice ruled out. The latter seems unlikely, however, since whole

litters came down when inoculated with the mosquito suspensions. Failure to kill chicks indicates they are not EE or WE. Suckling mouse neutralization tests on one strain ruled out EE, WE, and SLE.

Table 2. Seasonal distribution of EE and WE virus isolations from mosquitoes, South Alabama study area, November 1959 - December 1960.

	1959			1960								
	November			August			September			October		
Mosquito species	EE	WE	ratio infected	EE	WE	ratio infected	EE	WE	ratio infected	EE	WE	ratio infected
C. melanura	4	2	6/307	3	2	5/294	1	2	3/260			0/80
An. crucians			0/453	1		1/1349			0/673			0/387
C. nigripalpus			0/105	1		1/397		1	1/617			0/273
C. salinarius		1	1/35			0/436			0/43			0/71
A. vexans	1		1/69			0/13			0/19			0/5
M. perturbans			0/3	1		1/454			0/207			0/7
23 other species			0/344			0/460			0/702			0/271
Total EE	5			6			1			0		
Total WE		3			2			3			0	

November 1960

Mosquito species	EE	WE	ratio infected
C. melanura	1	3	4/291
An. crucians			0/1000
C. nigripalpus			0/1087
C. salinarius			0/902
A. vexans			0/111
M. perturbans			0/0
23 other species			0/412
Total EE	1		
Total WE		3	

REPORT FROM DR. DONALD D. STAMM
CHIEF, VETERINARY RESEARCH UNIT

The objectives of the wild bird study in South Alabama are to quantitate as many factors as possible in the avian part of the transmission cycle of encephalitis viruses in nature. Mist-nets are set up in a standardized pattern and operated on a standard schedule. Birds are banded, bled from the jugular vein, and released. Analysis of rate of capture and proportion of banded birds captured permits estimation of population density and provides data on population dynamics and movement of individual birds.

Only small amounts can be bled from small birds if maximal survival is desired. Most of the samples obtained are 0.2 to 0.5 ml of blood diluted to 1 ml volumes in the field. They are inoculated without further manipulation for isolation and then diluted to a final concentration of blood 1:10, centrifuged, and poured off. This final 1:20 dilution of plasma is used in serum-neutralization tests. To date, we have no test which will dependably demonstrate antibody to WE and SLE viruses in this kind of sample from birds known to have circulated these viruses. The intracerebral SN test in three week mice routinely used to demonstrate EE antibody is barely sensitive enough because some birds have been found to revert from positive to equivocal or negative within a few months. Plaque reduction SN tests in duck embryo tissue culture monolayer are being evaluated and may be the more sensitive system required. Laboratory tests on blood samples reveal the percentages of birds with viremia and immunity from previous exposure. Successive samples from individual birds provide a further measure of rate of virus transmission based on the number of conversions from negative to positive per susceptible bird per month.

Four isolations of EE virus from 413 bird-blood samples taken between November 17, 1959, and January 2, 1960, and no isolations from 668 samples taken between January 7 and February 27, were mentioned in issue number 1 of this newsletter. EE serum neutralization screening tests against 1 dilution of virus reveal that 60% of the permanent resident and 40% of the winter resident birds bled during this period were positive. Quantitative tests will be run simultaneously on all samples from birds bled more than once.

Additional 12-day periods of netting were conducted in May, July, September, and November, 1960, and about 2,000 more blood samples obtained. No virus was isolated in suckling mice from 600 samples

collected in May nor from 200 of the 500 samples collected in July. Seven isolations of EE and two of WE virus have been made from 100 of the 700 samples collected in September. Two isolations of EE and one of WE have been made from 90 of the 220 specimens collected in November. The remaining 730 samples from September and November will be tested simultaneously in suckling mice and duck embryo monolayer tissue cultures. It will be interesting to see whether a similar proportion of these contain virus. Preinfection or convalescent samples or both were obtained from most of the birds from which virus was isolated. For example, a specimen from a white-throated sparrow obtained December 29, 1959, yielded EE virus. This bird was bled again on January 10, February 25, November 15, and November 19, 1960. WE virus was isolated from November 15 sample. Many series of bleedings are on hand from birds captured as often as 12 times during the course of a year. These series will be used in evaluating serologic tests, determining patterns of rise and fall of antibody, and assessing cross-reaction between agents and response to re-exposure.

An attempt will be made to relate virologic findings to the five different types of vegetation present in the study area. The spot in which a bird was captured will not be useful for this purpose because individuals caught 7 to 10 times were captured in nets in all 5 types of vegetation. This points up a fundamental deficiency in the data gathered to date. We know a lot about where birds are and what they are doing during the day, but almost nothing about where they spend the night. Nighttime is critical in regard to virus transmission because exposure almost surely nearly always occurs at night. (This is based on the assumption that small birds would eat mosquitoes which attack them during the day.) To obtain this information, small radio transmitters, ultra-violet light activated colors and infra-red sensitive "snooperscope" devices have been considered, but all of these appear to be technically infeasible. One technique being tried on a small scale seems promising. Birds marked with reflective liquid ("Codit" - MMM Co.) can be located with a blue-filtered spotlight. We would welcome other ideas and suggestions on how to obtain this kind of information.

REPORT FROM DR. TELFORD H. WORK, CHIEF VIRUS & RICKETTSIA SECTION

The second issue of this newsletter carried a report of preliminary results of a serological survey of HI antibodies to certain arthropod-borne viruses in sera collected from Seminole Indians on Brighton and Big Cypress reservations in southern Florida. Further investigations toward establishing specificity of these reactions has brought out the difficulties in interpreting results of serological surveys by application of additional serological techniques.

It will be remembered that these sera were collected in the latter part of April, 1960. The sera were stored in glass vials at +4°C continuously until testing. Fifty-seven per cent of the Big Cypress Reservation and 7% of the Brighton Reservation sera inhibited hemagglutination by VEE virus sucrose acetone extracted VEE virus antigen.

In September and October a selection of VEE HI positive and negative sera were subjected to mouse neutralization test in the New York Laboratories of the Rockefeller Foundation. The first test was IC into adult mice with an LD_{50} dose of 125. None of 32 HI positive sera gave full protection. Only eight of 21 HI positive sera were positive in an IP 90 LD_{50} test in adult mice.

In the Arthropod-borne Virus Studies Unit at CDC, these sera were submitted to re-examination by NT using undiluted serum against five 10 fold dilutions of VEE virus diluted in fresh whole rhesus monkey serum. Thirty-seven of 37 HI positives so far tested had neutralization indices ranging from 2.2 to 4.0 logs with a mean of 3.09. When compared to sera drawn from persons who suffered laboratory infections with VEE virus five or more years ago (NI range 2.9-3.75) the results appear comparable and establish as clearly as can be established that Seminole Indians of southern Florida have indeed suffered infection with VEE virus in the past.

All the Seminole sera were HI screened against EEE and WEE viruses. The antigens were made from high mouse passage strains used in the RFVL and other laboratories for many years as reference strains. It may be recalled that there were surprisingly few positives to either EEE or WEE among Indians of either reservation, Brighton being near the locality where both EEE and WEE viruses have been isolated. In additional HI tests of the low titre EEE and WEE positive sera to determine the meaning of such low titres, a recent EEE strain isolated by Dr. Downs in British Guiana and 2 strains of HJ virus isolated by Taylor and Henderson in Florida were

used as antigen controls and convalescent sera from Massachusetts cases of EEE obtained from Mrs. Joan Daniels of the Massachusetts Department of Public Health laboratory were used as serum controls. Surprisingly, the convalescent sera did not react with the standard EEE strain but showed titres of 1:80 to 1:160 with the low passage recent EEE isolate from British Guiana. The Seminole sera must therefore be retested against the more significant antigen before conclusions can be drawn about the incidence of EEE virus infection in man in southern Florida.

By the evidence it appears that the HI test is a useful screening procedure when antigens of known significance are used. Confirmatory neutralization tests for specific antibody require consideration of the labile factor in sera tested more than two months after collection.

REPORT FROM DR. J. O. BOND
FLORIDA STATE BOARD OF HEALTH

Only 41 cases of eastern equine were reported in horses during 1960 and only 2 laboratory confirmed cases in humans. These were both non-fatal cases of eastern equine, occurring during January and February, and confirmed by four-fold titer rises against CF antigens.

REPORT FROM DRS. R. M. TAYLOR AND J. R. HENDERSON, THE DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH, YALE UNIVERSITY SCHOOL OF MEDICINE

Second Progress report on arthropod-borne virus survey in Florida

This survey was referred to in the two previous newsletters. Newsletter No. 1 briefly stated the reasons for undertaking this project and in the second newsletter a tentative list of the arthropods and vertebrates collected was given, together with reference to certain virus isolations.

This report will deal with additional laboratory work on the specimens collected with special reference to the classification of the virus isolates and the immunological reactions observed in bird plasma.

The following table shows the number of specimens collected, the number examined for virus and for antibody and the number of virus isolations:

Specimen	No. Collected	No. Examined for Virus	No. Examined for Antibody	No. Virus Isolations
Bird Plasmas	572	563	555	4
Mosquito Pools	452	449	0	10
Tick Pools	5	5	0	1
Sentinel Mouse Tissues	7	7	0	1
Sentinel Chick Plasmas	45	24	42	0
Rodents (Tissue)	143	126	0	0
Rodents (Plasma)	51	49	50	0
Reptile Bloods	9	9	2	0
Totals	1284	1232	649	16

There were 23 species among the birds netted, but influenced, no doubt, by most of them being netted at a long-established feeding station, blue jays and grackles accounted for about two-thirds of the total.

Twenty-two species of mosquitoes were recognized among the over 9,000 captured, but in the process of freezing and shipping the identifying scales were not infrequently scuffed off which prevented species separation of many of the Culex genus, thus Culex spp. group included the largest number of specimens followed by Anopheles crucians, Culex nigripalpus,

Mansonia perturbans, Psorophora confinnis and Uranotaenia sapphirina. These six groups comprised more than 80% of the catch. There were only 86 specimens of Culiseta melanura, the species which elsewhere has been commonly associated with the transmission of EEE.

It will be noted that 16 virus isolations were made, four from bird plasmas, ten from mosquito pools and one each from ticks and sentinel mice.

One of the isolates from bird plasma (purple grackle) and two from mosquito pools were identified as EEE. The mosquito pools from which this virus was isolated were comprised of Culex salinarius and Culex fatigans. One isolation of WEE was made from a pool of Aedes taeniorhyncus. A virus rather closely resembling WEE but in comparison with a WEE strain probably representing the original prototype isolated by Meyer, it appeared to differ sufficiently to warrant a separate classification and was tentatively designated Highlands J virus. However, subsequent examination of this strain in comparison with more recently isolated WEE strains cast some doubt on the validity of a separate classification and it may represent only a variant of WEE. Be that as it may, the HI reactions of the bird plasma with an antigen prepared from the HJ strain differed significantly from the reactions obtained with an antigen prepared from the WEE prototype strain (see following table). If this HJ strain is eventually regarded as a WEE variant, the reactions obtained in the bird plasma clearly illustrate the importance of antigenic characteristics of the particular strain utilized in surveys if the results are to be comparable. The characteristics of this HJ virus are still under study. It was isolated on three occasions, twice from bird plasma (Blue Jays) and once from a sentinel mouse.

In addition, there were isolated 8 as yet unidentified viruses which produced plaques in duck embryo tissue culture but which failed to consistently produce paralysis and death in infant mice. These viruses are likewise still under study. While the collections began the first of May and extended through to the latter part of July, all of the virus isolations save one in May were made in June and July.

Tissue culture utilizing several types of cells, including chick and duck embryo, monkey and hamster kidney were employed for virus isolation. The duck embryo tissue culture plaque method proved to be definitely more sensitive than infant mice for the isolation of EEE and the HJ strain as well as for all the unidentified viruses. Isolation of EEE and HJ in mice was possible only if the plaque count exceeded 100.

The following table gives the results of HI reactions with WEE, HJ, EEE, and SLE antigens, alone and in combination:

HI REACTORS WITH 420
SINGLE BIRD PLASMAS

NO. PLASMA REACTORS WITH ANTIGENS

Plasma Dilution	WEE	HJ	EEE	SLE	WEE-HJ	WEE-EEE	HJ-EEE	WEE-HJ-EEE	SLE and Gr. A
1-40	0	16	15	2	5	1	2	14 15 5 11	6
1-80	0	6	6	6	4 1		9	4 7 2 7	7
1-160	0	4	9	2	1 2	1	5	2 4 11 3	1
1-320	0	4	1	1	4		3	3 6 3	2
1-640 or greater	0	3	3	3	3		1	3 8 8	2
Total	0	33	34	14	10	1	20	32	18

As shown in the above table, 34 reacted singly with EEE, 33 with HJ, 14 with SLE and none with WEE alone. In combination, the largest number (62) reacted with HJ, 53 with EEE, 43 with WEE and 18 with SLE and an A Group antigen. Thus, the total number reacting singly and combined were with HJ antigen 95 or 22.5%; EEE, 87 or 20.6%; WEE, 43 or 10.2%; and SLE, 32 or 7.6% of the 420 bloods examined.

It seems quite evident that many of the combined reactions with viruses of the A Group, i.e., WEE, HJ and EEE are group rather than type specific.

Since in these associated reactions the titer of the serum is consistently higher with HJ than with WEE antigen, and usually higher than with EEE, it is not improbable that infection by HJ virus was responsible for most of these group responses. This concept is supported by the examination of 57 paired bloods collected from birds netted at one and two month intervals and finding that the conversion rate from negative to positive was highest (21%) with HJ antigen.

Contrary to what was found in birds, the ratio HI positive in 50 rodent sera taken mainly from cotton and black rats, was higher (30%) for SLE than for HJ virus (10%).

The results of this study to date may be summarized as follows:

1) The presence of EEE and WEE was established by isolation of both viruses from mosquitoes and EEE virus from a grackle, and supported by serological (HI) responses in birds.

2) The probable presence of SLE, or some closely related B group virus, was implied by HI antibodies in birds, 7.6% positive, and in rodents, 30% positive.

3) The isolation of a virus, twice from Blue Jays and once from a sentinel mouse, which though closely related to WEE may differ sufficiently from a prototype WEE strain with which it was compared to warrant separate classification. It is still under study but has been tentatively designated as Highlands J virus.

4) As the field collections were made at a time when there were few if any migratory birds present, it must be concluded that these viruses, EEE, WEE, HJ, and on serological grounds SLE, were being transmitted and are probably endemic within the area surveyed.

5) While some evidence was gained incriminating birds and possibly rodents as vertebrate hosts, and certain mosquitoes as vectors, a more intensive and prolonged study is required for a better understanding of the ecology and manner of maintenance of these viruses within the state.

6) Eight as yet unidentified agents were isolated by the TC plaque method, one from a nestling waterturkey (Anhinga) and seven from mosquitoes are now under study.

7) It is the opinion that the results of this survey have been sufficiently provocative to warrant more comprehensive studies on arthropod-borne viruses in Florida.

Surveillance of EEE in Connecticut

Field and laboratory studies on the ecology of EEE in Connecticut were conducted during 1960. Mosquito collections were hampered by extensive DDT control campaigns. As yet no virus has been isolated from the 4,730 adult female mosquitoes collected. A new study site has been established at Bradley Field, Connecticut, for the purpose of monitoring EEE activity by sampling blood and tissue from the indigenous crow population. Surveillance reports have indicated that EEE was not active in pheasants, horses, or man during the year.

Experiments on Interference

Using Mayaro and Sindbis viruses in primary chick embryo tissue cultures, it was found that interference was influenced by: 1) the infection

multiplicity of Mayaro interfering virus (infection interference) and, 2) the production of an inhibitor by Mayaro-infected cells (inhibitor interference). Inhibition of Sindbis plaque formation could be demonstrated when sodium desoxycholate-treated Mayaro virus was used as the interfering agent. When inactivated by heat, ultraviolet irradiation or Mayaro hyperimmune serum Mayaro virus was not found to interfere with Sindbis plaque formation.

REPORT FROM DR. M. MICHAEL SIGEL, DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF MIAMI SCHOOL OF MEDICINE CORAL GABLES, FLORIDA

A. Basic studies with dengue virus in tissue cultures.

1. A carrier system of dengue virus in KB cells has been developed and maintained for 24 months. The system is associated with several interesting properties, including resistance to superinfection with homologous and heterologous viruses.

2. Under certain conditions the cells of the carrier system undergo clumping in the presence of dengue immune serum.

3. Dengue virus was adapted to produce cytopathogenic changes in tissue culture.

B. Serologic tests were carried out on a group of sera collected in Cuba and sent to us by Dr. Herald Cox of Lederle Laboratories. Results shown in the following table resemble data obtained by us with sera of South Florida residents, especially as regards age distribution or reactors.

Source of Sera	Age in Years	Number tested	Number positive	No. with titer* of			
				Neg.	10-20	40-80	160 or greater
Mantanzas City	7-12	15	0	15			
Camaguey City	6-12	15	0	15			
Aquacate Town	7-12	14	1	13	1		
Havana City	19-49	20	10	10	2	3	5

*Reciprocal of HI end-point

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

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

The activities of this laboratory are conducted at the Middle America Research Unit in the Panama Canal Zone and at Bethesda. The Middle America Research Unit is a joint research effort of the National Institute of Allergy and Infectious Diseases which has cognizance for studies on virus diseases and the Walter Reed Army Institute of Research which has cognizance for studies on fungus diseases.

Virus Isolates from Panamanian Mosquitoes and Sandflies

During the first 12 months of a 3-year project on the ecology of arthropod-borne viruses in the tropical rain forest, conducted by the Gorgas Memorial Laboratory with the collaboration of MARU, major emphasis has been on virus isolation in suckling mice and hamster kidney cell cultures. Fourteen virus strains were isolated at MARU from 412 pools and 63,000 specimens provided by the Gorgas Memorial Laboratory. Virus isolation rates were for Phlebotomus, 1:700 and for mosquitoes, 1:7000, although the rates varied greatly with species. Of the five Phlebotomus isolates, two of broad host range (including cell culture) and short incubation period are serologically identical. These viruses have now been identified as the Indiana type of vesicular stomatitis virus. The other three Phlebotomus and nine mosquito viruses are being compared to each other, to known virus groups, and to human and/or animal infection and disease.

Eastern Equine Encephalomyelitis Virus Infection in Panama

The prevalence of EEE antibodies in horses and man in two areas of suggested EEE virus endemicity has been determined, allowing an evaluation of the relative usefulness of several serological methods applicable to studies of this type. It was found that the incidence of EEE antibodies in 460 humans tested increased with advancing age (0.8% under 10 years with progressive increase to 9% in the 41-50 year group). Complement fixation results on the same sera indicated the probable presence of other group A viruses.

Lizards of species common to this part of Panama were examined as a possible virus reservoir. Specific EEE virus hemagglutination-inhibitors were found in some of their sera. The occurrence of viremia

and HI antibody response following virus inoculation were experimentally confirmed by inoculation of lizards.

Encephalomyocarditis Virus Infection

Previously this laboratory described an outbreak of a fatal disease of swine caused by the EMC virus. The outstanding lesion in pigs dying during the outbreak was acute myocarditis. Since epidemiological observations suggested that natural infection resulted from ingestion of contaminated food, experiments were undertaken to reproduce the disease by feeding virus to young pigs. Viremia and virus excretion from the gastrointestinal tract were found to occur following the administration of brain from EMC inoculated mice. Infected pigs developed high titers of HI and neutralizing antibody during convalescence and had myocardial fibrosis at autopsy. Other studies included demonstration of EMC antibodies in a small number of city rats and rats caught on the affected farm, although wild rodents were found to be negative. Human sera were examined with interesting differences in the results depending on the donor's age: while a substantial proportion of the Panamanian population has been infected with EMC virus, the antibodies were found to be more common in persons of younger age.

Enterovirus Flora in Children of Central America

For a period of 12 months the enterovirus flora of infants at an outpatient clinic in Panama City was systematically explored, establishing a base line of enterovirus fluctuation. The majority of viruses isolated belonged to the ECHO group, although in late 1959 and early 1960 poliovirus type 2 had become very prevalent. This was reflected in an uncommon occurrence of a small outbreak of paralytic disease due to type 2 poliovirus.

Other enterovirus studies have included 1) surveillance for the presence of type 1 poliovirus in Panama in late 1960 as a check on dissemination and threatened spread of this commonly epidemic type, 2) studies on a major epidemic of ECHO-9 virus which swept through the Republic of Panama and the Canal Zone, and 3) initiation of a collaborative project on possible relation of enterovirus flora of Guatemalan children to their dietary status.

REPORT FROM DRS. PEDRO GALINDO AND ENID DE RODANICHE, GORGAS MEMORIAL INSTITUTE OF TROPICAL AND PREVENTIVE MEDICINE, PANAMA, REPUBLIC OF PANAMA

Summary:

During the year 1959-1960 investigations of the ecology of the arthropod-borne viruses in Panama were continued. Attempts were made to isolate viruses in suckling mice from human and animal blood and from diptera captured in the forest canopy and on the ground. Steps toward the identification of these viruses, within the limits of the facilities of the laboratory, also were taken. Furthermore, serological surveys for antibodies against certain of these viruses, with special attention to yellow fever, were conducted with the blood of humans and wild and domestic animals. Three field projects were conducted during the year: The Almirante Project, the Cerro Azul Project and the Tocumen Project with definite and distinct objectives, which will be discussed separately.

Almirante Project

This 3-year project is supported in part by Grant E-2984 of the National Institutes of Health and is being conducted by Gorgas Memorial Laboratory with the cooperation of the Middle American Research Unit, in the Panama Canal Zone, and of the United Fruit Company. Its objective is the investigation of the ecology of arthropod-borne viruses in an area with a well-defined tropical rain forest climate.

A total of 138,064 mosquitoes and Phlebotomus were taken during the year. These included about 40 species of mosquitoes, while the bulk of the Phlebotomus were of two species. From 604 pools of mosquitoes 9 viral agents were isolated at G. M. L. and from 476 pools, 8 viruses were isolated at MARU. Eighteen pools of Phlebotomus yielded 2 viruses at GML and 18 pools processed at MARU yielded 5 viruses. Of the 25 viruses obtained, 9 were from species of Psorophora (Janthinosoma) and 7 from Phlebotomus sandflies. Identification of viruses is still incomplete, though MARU has identified one of the Phlebotomus viruses, as vesicular stomatitis and GML has identified one of the Psorophora viruses as Ilheus encephalitis.

The animal bleeding program at Almirante yielded bloods from 1,306 mammals belonging to 37 species, 650 birds of 72 species, and 36 reptiles of 11 species. Virus of Ilheus encephalitis was isolated from blood of two of 116 bird sera so far tested, and antibodies against the same virus was found in the sera of 5 of 62 birds tested.

Cerro Azul Project

This project is being supported in part by Grant E-1941 of the National Institutes of Health. Its objective is the investigation of sylvan yellow fever in an area where the virus has been known to be active in two previous occasions (1948 and 1956).

A total of 8,992 mosquitoes, largely species of Haemagogus and Sabethines were taken and inoculated intracerebrally into mice for virus isolation. Four viruses were isolated, one from Wyeomyia spp. has been identified as St. Louis encephalitis, the other three are as yet unidentified. No yellow fever virus was recovered.

Tocumen Project

In relation with an outbreak of eastern equine encephalomyelitis in horses in the Tocumen area some 15 miles east of Panama City, a station was established there at the beginning of August 1959 and kept up for approximately one month. Mosquito catches at this station were mainly done at night using horse and chicken baits and light traps. Daytime collections were confined to the diurnal resting places of night-flying mosquitoes. Over 32,000 mosquitoes were captured and injected intracerebrally into mice. Two viral agents were isolated from these insects, one each from Culex nigripalpus and Culex amazonensis. Although these isolates remain unidentified they did not prove to be eastern equine virus.

Survey of Humans and Animals for Antibodies to SLE, YF, and Ilheus Viruses in Darien

In the early months of 1959 blood was taken from 195 residents of the town and settlements along the course of the Tuira, Pucro and of the Paya Rivers of Darien. Also 248 bloods were obtained from local mammals and birds. These were tested wherever the quantity of serum permitted against the 3 Group B viruses known to occur locally, Ilheus, SLE, and yellow fever. Of the human bloods, 8.4% neutralized 2 or more logs of St. Louis virus, 43.7% two or more logs of Ilheus virus and 42.0% two or more logs of yellow fever virus. The high proportion of positive reactions against Ilheus virus was surprising to us as Ilheus infection has not been recognized clinically in Panama. Further investigation is indicated. In the case of yellow fever, it was not possible to determine whether the presence of antibodies was the result of natural infection or artificial immunization. However, the result indicates that only about half of the rural population in this area is demonstrably protected against yellow fever although this is the group most exposed.

The 248 animal bloods mentioned were tested against St. Louis encephalitis, yellow fever, and Ilheus encephalitis. Positive reactors in both birds and mammals were found with SLE, five birds gave positive results with Ilheus, and two bird and two mammal bloods gave weak reactions with yellow fever. This work is now in press.

Continued survey for yellow fever antibodies in captured and purchased animals yielded only one positive, an adult Cebus monkey from Darien, indicating the continued inactivity of the virus in Panama.

Identification of Viruses Isolated in Previous Years

Work was continued where time permitted towards the classification of viruses whose isolation was recorded in previous Annual Reports. A strain of virus recovered from Wyeomyia spp. in Darien in June 1958 was identified as that of St. Louis encephalitis. Also two viruses obtained respectively from Trichoprosopon spp. captured in Darien in April, May, and June 1958, and Haemagogus spegazzinii falco captured at Cerro Azul in June 1958 were found to be immunologically closely related to Ilheus. As these isolations of Ilheus virus represented the first in Panama, they have been reported for publication. (Isolation of the virus of Ilheus encephalitis from mosquitoes captured in Panama, Rodaniche, Enid de and Galindo, P., In Press.) The group of 7 viral agents recovered from Culex dunni captured in Pacora in October and November 1958 have been found to belong to at least two different immunological groups.

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

The major events of 1960 centered about the Bush Bush Forest program, started in late 1959, and productive of viruses both in numbers and in variety beyond our most sanguine expectations.

The Bush Bush Forest location, in the middle of the Nariva Swamp in east Trinidad, was selected in hopes of getting close to the home territory of the yellow fever virus. Yellow fever virus has not been picked up since this Bush Bush program was started. Venezuelan equine encephalitis virus, undetected in Trinidad since isolations in 1943, appeared to be common in this region, with 93 isolations from sentinel mice, 14 from mosquitoes and 12 from trapped wild rodents of three different genera. Agents of the Guama group, this group newly recognized by work of the Belem Laboratory, have been collected with regularity, there being 46 isolations from sentinel mice, 2 isolations from wild rodents and, to our mystification, no isolations from wild mosquitoes. The surprise is heightened by the fact that the first (and until the Bush Bush program the only) Trinidad isolation in this group,

TRVL 8362, was made from mosquitoes in 1955. One of the recent isolations in the Guama group is closely related to the Brazilian virus 'Catu'. Guama group agents have been recovered from human fever cases in Brazil. Thus far we know nothing of their human disease potential in Trinidad.

The Belem workers have been studying viruses of a Group C complex for years. Failure to isolate such agents in Trinidad has been a challenging fact. The Bush Bush Forest work has yielded in 1960 numerous isolations of a virus (possibly more than one agent) belonging to Group C, and related to, but quite probably not identical with the Brazilian viruses 'Apeu' and 'Caraparu'. There have been 26 isolations from sentinel mice, 2 isolations from mosquitoes and 1 isolation from a wild rodent of the agent(s). Since Group C immunity has already been demonstrated in a small sampling of Trinidad residents, a fertile field for study has been opened.

The Bush Bush Forest location also yielded two isolations of Ilheus virus from mosquitoes. This virus, picked up repeatedly in 1954, 1955, 1956, and 1957, was not encountered in 1958 and 1959. The 1960 isolates indicate that it is indeed still with us. A strain of St. Louis virus was isolated from mosquitoes in Bush Bush.

The Vega de Oropouche location, worked for many years, was operated part time and yielded two isolations of eastern equine encephalitis virus, and 6 isolations of St. Louis encephalitis virus, five from mosquitoes and one from a bird (Pipromorpha oleaginea). Isolations of EEE in Trinidad thus far have only been made from mosquito pools composed of Culex species (C. taeniopus and C. nigripalpus) from chicken-baited mosquito traps. EEE immune rates approaching 10% have been encountered in wild birds from eastern Trinidad. Certain species of wild birds, the Orange-headed Manakin (Pipra erythrocephala), the Black and White Manakin (Manacus manacus) and the Bare-eyed Thrush (Turdus nudigenis) have higher immune rates than their congeners.

Studies on distribution of immunity to the arthropod-borne viruses in this part of the world have continued, with a survey and serum collecting trip to Surinam in January 1960, and with continuing laboratory analyses of specimens collected in earlier years from other West Indian islands and British Guiana, Venezuela, and Curacao. A special job was done on serum specimens submitted from Jamaica, indicating that Group B virus immunity in Kingston residents is largely immunity to dengue and in the rural regions, largely immunity to SLE.

The year 1960 in Trinidad would appear to be more of a "virus year" than the several years preceding it. This point is difficult to maintain in an absolute fashion, due to uncontrolled factors such as shifts in venue of program. The year was somewhat drier than average but considerably wetter than 1959 (81 inches as against 70 inches - overall average for east Trinidad). There is certainly increased evidence of virus activity during the rainy season, presumably linked with increased mosquito production. However, our procedures are as yet not standardized enough to permit comparing virus isolations of one rainy season with another. Continuing observations may eventually yield good information on this point.

A pilot study to familiarize laboratory personnel with basic procedures in isolation and identification of enteroviruses in tissue culture yielded 14 strains of Poliovirus I plus assorted viruses as yet unidentified.

The tissue culture section made significant advances in development of technics for isolation of arthropod-borne viruses from human cases, animals, birds and insects. Virus neutralization tests in tissue culture have been shown to be satisfactory for some viruses and it is hoped that for certain viruses (VEE, EEE, WEE, Mayaro as a start) such tests may take the routine load of neutralization tests off the mouse colony.

Plans have been completed for moving the laboratory from the present converted Army barracks on Wrightson Road, Port-of-Spain, to the modern laboratory formerly operated by the Colonial Microbiological Research Institute, in Federation Park, Port-of-Spain. This laboratory was given to the Government of Trinidad and Tobago by the British Government (buildings) and the Government of Trinidad and Tobago is making buildings and lands available to the University College of the West Indies. The UCWI in turn is making the laboratories available to the Trinidad Regional Virus Laboratory. It is necessary to construct a large new one-story building for animal colonies, and additional office space for professional staff, and much internal modification of existing laboratory rooms will be necessary. Funds for this work have been granted by the Rockefeller Foundation. It is hoped that the laboratory will be in operation in new quarters by July 1961. Arrangements are also being made for the laboratory to be included in the University College of the West Indies to form a graduate extension arm of the Department of Microbiology of the Medical School, located in Mona, Jamaica.

Personnel changes during the year include the addition of two Rockefeller Foundation staff members, Dr. C. Brooke Worth and Dr. Andries Jonkers, and one post-graduate fellowship holder, Dr. Elisha Tikasingh. Dr. Edward A. Belle, formerly on fellowship has gone to the University College of the West Indies (Department of Microbiology) for a year.

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

Summary of Activities and Results, 1960:

During this sixth year of activity in the Belem Virus Laboratory more isolations of virus were made, processed, and identified, than in all the previous five years. A total of 840 isolates of filtrable agents including 58 non-arborviruses, was studied in 1960. Some of the 782 arbor viruses are actually duplicate or multiple isolations of the same virus from one host or from the different individuals in one sentinel mouse group, and in these instances probably represent a single infection. Thus for epidemiological or frequency tabulations the number of infections, rather than number of isolations, have been recorded. Including the 13 isolations in November and December 1954, and the 81 non-arbor viruses agents also processed, the total number of isolations in the Belem Laboratory stands as 1610 at the end of 1960. The total number of infections is 720.

An epizootic in which Eastern equine encephalomyelitis (EEE) virus was isolated from horses and mosquitoes in the Braganca region and on outbreak of encephalomyocarditis (EMC) virus apparently erupting simultaneously over a wide area during a limited period, were events during the first third of the year. The first isolation of yellow fever virus since 1955, from a sentinel monkey on the Brasilia Road, the isolation of St. Louis virus from Gigantolaelops sp. (rat mite), as well as from Sabethes belizarioi and sentinel mice, the isolation of three other as yet unclassified viruses from Marmosa and Nectomys and man, the recognition of a new type in group C (AN10655) and two additional group Guama agents (AN20525, AN20076), and the finding of the TRVL virus Oropouche from Bradypus tridactylus (sloth) and Aedes serratus, were notable firsts during the last two trimesters.

The outstanding achievement in serology is the preparation of hemagglutinating antigen for the group Guama agents. This has resulted in recognition in the group of at least one new complex (Capim, Guajara, AN20076) and has associated with it another previously ungrouped virus (AN7722). These with a new Guama agent differentiated by CF testing (AN20525) and Trinidad's Bimiti and TR26668, bring to at least nine the number of distinct serological types so far identified in the Guama group, which has more known types in the Amazon region than any other group.

Ecological information has been assembled through continuous sampling by sentinel mice in the IAN forest. Results obtained from observations on these sentinels suggested a definite periodicity in

general virus transmission, which is strikingly inverse to the accumulated total precipitation per month. In other words, less virus infections occur during periods of heavy rainfall than in months with lower total precipitation. However, there appears to be a critical amount of rainfall which is required for support of virus activity, and below which virus transmission is inhibited. Thus a period of relative drought, when total precipitation was less than 100 mm. per month, coincided with the fall-off of virus infections in October and November 1960. However, immunization of the animal population during a period of exceptionally high transmission rates, might also reduce transmission during subsequent months even in the presence of a sufficient number of infected vectors.

Antibody studies have been made during the past two years on more than 2300 wild animal sera, most of them from IAN forest collected during the past six years and made available by Dr. Hugo Laemmert of the Oswaldo Cruz Institute. This represents over 26,000 separate determinations, which, if broadly interpreted, give evidence for the presence of infection in these animals by VEE, EEE, Mayaro, Bussuquara, Ilheus, yellow fever, Oriboco, Murutucu, Caraparu and Tacaiuma. HI antibodies of Bussuquara and group C viruses have not shown significant fluctuation in animals from year to year. On the other hand VEE virus seems to have been active during or before 1955 and 1956, then to have nearly disappeared. In 1958 there was only a very low rate of residual antibody. In 1959, however, antibody reappeared. There is thus evidence that a complete cycle of VEE activity may have occurred in the study area forests during this five-year period, which is also reflected in records of VEE isolations.

During the past year, 1305 human sera from the Amazon Valley have been surveyed for HI antibodies against a battery of 17 arbor viruses isolated in Brazil. The data accumulated (over 21,200 separate determinations) presents evidence for the widespread presence of infection in man by VEE, Mayaro, Una, yellow fever, Ilheus, Caraparu, Cache Valley, Guaroa and Kairi, and in localized areas by EEE, Murutucu, Oriboca, and possibly Aura.

The accompanying table shows the names of viruses isolated, the Belem prototype number, and the source of isolation during the past six years. Twenty-eight of these types were obtained in 1960 either as reisolations or new isolations in Brazil. The agents which were first isolated in the Belem laboratory are underlined.

NAME OF VIRUS, BELEM PROTOTYPE AND SOURCE OF ISOLATION FOR PERIOD 1954 - 1960

VIRUS			SOURCE			
Group	Type	Belem Prototype	Human	Animal		Arthropod
				Sentinel	Wild	
A						
	VEE	AN10967	+	+	+	+
	EEE	AN7526		+*		+
	Mayaro	H407	+			+
	Aura**	AR10315				+
	Una	AR13136				+
B						
	Yellow fever	H111	+	+		+
	Bussuquara	AN4116		+	+	+
	Ilheus	H7445	+	+		+
	St. Louis	AR23379		+		+
C						
	Oriboca	AN17	+	+	+	+
	Murutucu	AN974	+	+	+	+
	Marituba	AN15	+	+		
	Apeu	AN848	+	+		
	Caraparu	AN3994	+	+		+
	Itaqui	AN12751	+	+	+	
	AN10655	AN10655		+		
Guama						
	Guama	AN277	+	+	+	+
	Catu	H151	+	+	+	+
	Moju	AR12590		+		+
	AN20525	AN20525		+		
	Capim	AN8582			+	
	Guajara	AN10615		+		
	TRVL26668	AN20076		+		
	AN7722			+		+
Bunyamwera						
	Guaroa	H12208	+			
	Cache Valley	AR7272				+
	Kairi	AR8226		+	+	+
	Wyeomyia					
	Tucunduba	AR278				+
	Taiassui	AR671				+
	AR8933	AR8933				+
	TRVL9375	AR8033				+

VIRUS			SOURCE			
Group	Type	Belem Prototype	Human	Animal		Arthropod
				Sentinel	Wild	
Simbu						
	Oropouche	AR19886			+	+
Ungrouped						
	Tacaiuma	AN73		+		+
New agents						
	H22511	H22511	+			
	AN24232	AN24232			+	
	AN24262	AN24262			+	

*also from horses in epizootic
**underlined agents were first isolated in the Belem Virus Laboratory

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

Phlebotomus fever:

Stimulated by the successful isolation of phlebotomus fever virus from wild-caught Phlebotomus flies collected in the summer of 1959, search for virus in wild-caught flies continued in 1960. Fourteen isolates were recovered from 82 pools comprising over 36,000 female Phlebotomus flies collected in the Cairo area between April and November 1960. Two of the isolates have been identified as the Sicilian type of phlebotomus fever virus and remainder await identification. Phlebotomus papatasi continues to be the only species collected from human dwellings in the area.

Near Eastern equine encephalomyelitis:

(In collaboration with the Ministry of Agriculture) Attempts to identify the etiologic agent of this sporadic fatal disease continued. The following progress has been made:

1) The demonstration that West Nile virus is probably not responsible for equine encephalomyelitis in Egypt. In spite of the valid but fortuitous isolation of West Nile virus from a fatal case and

a high incidence of West Nile neutralizing antibody in equines, susceptibility experiments indicate that donkeys and horses are quite refractory to infection with this agent. Animals inoculated intravenously with low passage virus did not develop a detectable viremia, showed no signs of overt infection, and produced only low levels of neutralizing antibody. Equines inoculated intracerebrally also failed to develop apparent infection. The high incidence and level of antibody in naturally exposed equines is considered to be the result of repeated antigenic stimulation with low non-infectious doses of virus.

2) Virus isolation has been attempted to date on central nervous system tissue and blood of 65 clinically diagnosed cases (57 donkeys and 8 horses). Two immunologically similar or identical strains of an unknown virus and one isolate of West Nile virus (see above) were derived from horse tissue; two other isolates, immunologically similar to each other but different from the horse isolates, were recovered from donkey tissue. The horse and donkey agents, initially virulent only for suckling mice, have been adapted to adult mice and are being used in surveys for neutralizing antibody in equine sera. The pathogenicity of the strains for horses and donkeys is under study.

3) Direct transmission trials suggest that the etiologic agent may not be viral in nature. None of four donkeys inoculated by combined intracerebral and peripheral routes with filtrates of freshly collected central nervous system tissue from typical donkey cases showed signs of infection. Further transmission attempts are being made to establish the nature of the causative agent.

West Nile fever:

The possibility that Argas ticks serve as an overwintering host for West Nile virus was suggested by a single isolation of this agent from a pool of Argas hermanni collected in January 1954 (Taylor). Additional evidence for the role of Argas ticks in the maintenance of the virus during winter months was obtained during the past year. West Nile virus was isolated from two of 28 pools of Argas hermanni collected in February, midway in the interendemic period. Experiments are being planned to determine the persistence of the virus in laboratory-reared ticks held under simulated winter conditions.

Arbor Virus Publications:

Schmidt, J. R., Schmidt, M. L., and McWilliams, J. G. Isolation of phlebotomus fever virus from Phlebotomus papatasi. Am. J. Trop. Med. and Hyg. 9:450-454 (1960).

Schmidt, J. R., and Kamel, H. Survey for New World equine encephalitis antibody in Egyptian equine populations. J. Egyptian Publ. Hlth. Assn., 23:135-138 (1960).

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

This program has included an extensive investigation into the biology, ecology, and taxonomy of vertebrates and their ectoparasites in Egypt as in adjacent countries, in the Middle East and Africa. Tremendous numbers of specimens and field and experimental data has been assembled. As result of this effort, the many reports published include monographic studies on mammals, reptiles, birds, ticks, fleas, lice and mites. Further examples are reports on ecology distribution, epidemiological relationships, spirochetal and bacterial infections, life histories of parasites and descriptions of new species.

During the past year considerable time was devoted by the chief investigator in inaugurating and putting in operation a research program on vertebrates in the kala-azar infected area in Southern Sudan. Satisfactory progress was made in the biological and systematic studies of ticks of the genera Haemaphysalis, Hyalomma, Boophilus, Argas, and Ornithodoros. A preliminary plan for species relationships and host-parasites relations in the tick genus Haemaphysalis was made. This will result in a revision and review of the genus. The phylogenetic aspect of this study was commenced as a background for the study of phylogeny of viruses. Considerable progress was made in the revision of the economically important genus Hyalomma. Data on distribution, ecology and host relations of Egyptian Hyalomma ticks have been collated. Extensive series were reared in the laboratory for comparative and crossing studies. Special attention was given to the H. marginatum rufipes complex. A new species of Boophilus from sheep and goats in Jordan was described. This discovery increases the number of species in this cosmopolitan and economically important genus of ticks by 25%. With the arrival of several lots of Argas ticks from different parts of the world the results obtained from comparative studies of their progeny criteria for recognizing this bird parasite was determined. As result, a generic revision on a world-wide basis started, and series of studies on Argas ticks of Western Europe, Africa, Northwestern United States, Peru, and Chili were published or are under publication. The bat-infesting

Ornithodoros ticks of the world were reviewed and a new species from the Belgian Congo was described. Also, a report on Ornithodoros coniceps from wild swallow nests in India was submitted for publication. Studies on the ticks of Yemen, Libya and Jordon were published or in the last stage of preparation. These are part of the series to supply the F. A. O. of the United Nations with a specific account of the ticks of the countries of North Africa and the Near East. Results of five years of effort in studying the bird migration in Egypt were collated and final reports were submitted for publication.

Our collaborators have made considerable progress in their fields of speciality during the reporting period. The Egyptian flea monograph is in the last stage of preparation and will be published early next year. Sections on gerbils, spiny mice, meriones, foxes and gazelles were published or nearing completion towards a monographic study of Egyptian mammal hosts. An outstanding monograph on "The Anoplura of African Rodents and Insectivores," based in great part on NAMRU-3 collections, was published. A study on the Egyptian Anoplura infesting rodents is under publication. Collections from domestic animals are being made for a similar study. Material and data of mites accumulated in past years are being studied and assembled.

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

Virus isolation processing of sandflies collected in West Pakistan and Iran during 1959 has been completed. One hundred fourteen lots containing 12, 656 sandflies have been processed and 39 virus isolates obtained. The virus isolate yield from sandflies collected in West Pakistan was as follows: 7 isolates from 37 lots (3, 691 specimens) of female Phlebotomus tested, 3 isolates from 10 lots (1, 728 specimens) of male Phlebotomus tested, 3 isolates from 15 lots (1, 590 specimens) of female Sergentomyia tested, and 1 isolate from 1 lot (34 specimens) of male Sergentomyia tested. The virus isolate yield from Iranian sandflies was as follows: 22 isolates from 40 lots (4, 123 specimens) of female Phlebotomus tested, and 3 isolates from 11 lots (1, 490 specimens) of male Phlebotomus tested. Ten of the virus isolates have been reisolated from the original sandfly inocula and the remainder are currently being processed for reisolation.

Identification of the viral isolates obtained from sandflies has been limited to one isolate from West Pakistan--identified as the Sicilian strain of sandfly fever. Identification of the majority of the isolates

has been hampered by low titers in the antigens, even in instances where 17 to 31 suckling mouse passages have been made. Hyperimmune rabbit sera prepared from some of the isolates have been unsatisfactory to date. However, it is apparent from neutralization test results that many of the isolates represent previously undescribed viral agents and some grouping has been accomplished by cross protection challenge tests in mice.

Eight virus isolates have been obtained from the sera of febrile patients having clinical syndromes consistent with that described for sandfly fever. Four of these were obtained from patients in West Pakistan and 4 from patients in Iran. Two of the isolates from Pakistan and two from Iran have been reisolated from the original inocula. Three of the viral isolates from Iran appear to be the Sicilian strain of sandfly fever while the fourth remains unidentified, and apparently is unrelated to either the Sicilian or Naples strains of sandfly fever. One of the Pakistan isolates from patients appears to be related to the Sicilian strain but the remaining three are unidentified.

The colony of Phlebotomus papatasii established at WRAIR with materials collected in Pakistan and Iran in 1959 was maintained for five generations and then died out. A new colony of this species has been established from material collected in the Peshawar area of West Pakistan in 1960 and appears to be better established than the original colony, although it is only in the third laboratory reared generation. A colony of Phlebotomus argentipes, the vector of kala-azar in the Indian subcontinent has also been established at WRAIR.

Experiments have been undertaken to determine the natural course of infection of Japanese encephalitis virus in horses and to determine the possible importance of horses in the natural dissemination of the virus. Fourteen proven infected Culex tritaeniorhynchus mosquitoes which had fed on a chick with a titer of 4.5 logs 23 days previously were fed upon an elderly horse. Four days later viremia commenced. LD_{50} titers in adult mice were as follows: 1.0 logs on day 4, 1.2 logs on day 5 and 0.5 logs on day 6. Virus could not be detected prior to day 4 or subsequent to day 6. On day 10 the horse developed fever, loss of equilibrium and continuously ground its teeth. The following day the horse had pronounced signs of encephalitis but it subsequently went on to recovery. Batches of both Culex tritaeniorhynchus and Culex gelidus were fed upon the horse daily, and those which had fed upon the horse during the period of viremia (days 4-6) were tested for their ability to transmit virus to young chicks, after extrinsic incubation periods varying from 25-40 days. Culex tritaeniorhynchus transmitted virus once in 59 attempts, but Culex gelidus failed to transmit in 52 attempts. However, a group of 85 C. tritaeniorhynchus which had fed on the horse on day 5,

transmitted virus to a second horse. It was determined subsequently that only 3 of the 85 mosquitoes which fed on the second horse were infected with virus. The second horse did not develop viremia until day 4 (titer 0.6 logs) and had a titer of 0.5 logs on day 5 and 0.7 logs on day 6. By day 7 it was aviremic and subsequently developed no signs of overt illness.

FROM THE ANNUAL REPORT OF THE VIRUS RESEARCH CENTRE, POONA, INDIA

1. INTRODUCTION

The highlight of the year's activities of the VRC was the participation in the study of the epidemic of African horsesickness which was first recognised in April. The virus isolated from the epidemic among cavalry horses at Jaipur was identified as African horsesickness. The epidemic spread widely in Northern India, involving at least nine states. Vaccination was started in August and by the end of the year reports of activity of the virus had decreased greatly.

The inquiry into Kyasanur Forest disease (KFD) in Shimoga District of Mysore State has continued to receive the major proportion of the laboratory and field effort. The tick infestation of birds, monkeys, and humans has been intensively studied. Testing of survey sera collected from birds and monkeys was begun. A determined effort to locate the source of virus for the larval ticks was launched.

The field station at Vellore in the Christian Medical College has continued the study of virus infections in that area. Three of the viruses isolated late in 1959 have been identified as strains of dengue virus. With the assignment of a professional entomologist to the laboratory it has been possible to begin many of the investigations that have had to be delayed for want of such a person. Both the entomological and virus facilities have been considerably expanded during the year with the result that it will now be possible to increase very significantly the activity of the field station.

One of the principal objectives of the VRC has been to train personnel. During the year, several people took advantage of the opportunities offered. Limitations of space plus the fact that construction of one sort or another has been in progress since 1957 has limited the amount that could be done. On the other hand, there have not been many applications and greater use could certainly be made of the facilities which are now available. It is hoped that the training program of the VRC will become an increasingly important function during the next few years. One of the chief 'bottlenecks' involves finding and stimulating the interest of capable graduates.

With the arrival of new equipment, the past year has seen the almost full utilization of the new building. As soon as the new building was completed in 1959, plans were drawn for the new entomological laboratories which will occupy the entire group floor of the old building. By the end of the year, the entomological laboratory was finished and equipped. Also, staff quarters were begun which will consist of four large apartments. The construction was supposed to have been completed by October but due to an extraordinary number of delays the building will not be complete before the first of April, 1961.

2. AFRICAN HORSESICKNESS INVESTIGATION

In May, a team from the VRC investigated an epidemic among horses of an Indian Army Cavalry unit in Jaipur, Rajasthan State. The disease which first appeared on April 21, 1960, had been diagnosed clinically as African horsesickness. The appearance of the disease in India was not unexpected since Pakistan had reported cases during the past year and there was news of infection in other Middle East countries. The team obtained blood samples from acutely ill horses from which virus was later isolated. Several strains were identified as African horsesickness virus at the Onderstepoort laboratory in South Africa. Although the viruses from India were not identical to any South African type, they were most closely related to S. A. type 6. Both whole heparinized blood and serum from many horses were tested simultaneously for virus and many more strains were isolated from whole blood than serum. Virus was isolated only during the first nine days of illness. Following the inoculation of blood or serum from infected horses, the incubation period was shorter and more isolations were made (15/15 to 10/15) in infant mice as compared to adults. Convalescent sera were found to contain high titre neutralizing (NT) and complement fixing (CF) antibodies. After the epidemic had subsided, a number of the remaining healthy horses were bled and their sera tested for the presence of CF antibodies. Only two of 66 horses and one of four mules had antibodies, which would indicate that the sub-clinical infection rate was low.

April and May were the hot, dry season in Rajasthan and it seemed at a distance unlikely that a disease spread by Culicoides would be making its first appearance at that time of year. Once on the site, it became clear why the disease could be present. The Cavalry Lines were situated near a nullah which was almost entirely under standing water varying in depth from a few inches to several feet. Tall grass, small trees and water hyacinth grew throughout. At least four species of Culicoides were captured of which C. oxystoma comprised 97% of the total. Activity studies revealed that the Culicoides were chiefly active at night, more particularly during the second half of the night. Evidence though was obtained that they might be active on cloudy days. They were found as frequently inside the stables as

outside and in that regard differed from the activity reported for the South African vectors. Culicoides were not taken biting man but blood engorged specimens were collected off horses. A total of 1767 specimens in 37 pools were tested for the presence of virus (some by Col. S. L. Kalra at the All India Institute of Medical Sciences in New Delhi and some at the VRC.) No virus was isolated.

In order to protect the horses from Culicoides and to break the transmission cycle the following measures were recommended.

1. The coating of the horses daily with dimethyl pthalate or as a second choice, D. D. T. wettable powder.

2. Use of fog generator to produce an aerial suspension of D. D. T.

3. Screening of sick horses with fine wire mesh.

4. Spraying of the nullah with D. D.T. and Malariol, and

5. Residual spraying of the stables.

The first of these measures was instituted May 28th and may have contributed to the break in transmission. The last case was reported on June 3rd.

Entomological observations were continued at Jaipur for some time after the epidemic subsided. It was thought as a result that the nullah was the principal breeding place for Culicoides. Many larvae were found in soil samples taken from a number of sites in the nullah. After the spraying of the nullah many of the original sites from which larvae had been recovered were retested. No larvae were encountered. Oddly enough, adults continued to be taken. It was conjectured that some places in the nullah failed to receive sufficient insecticide and continued to breed Culicoides.

Soon after the identification of the virus was made, and the alarm sounded, cases began to appear in other localities. Once the rains set in, an epidemic occurred. The presence of cases was finally reported from nine states and the last report received by this laboratory in November listed over 9,000 deaths. Vaccine was received in the country in August and the horse breeders in Poona vaccinated their stables immediately thereafter. The vaccine employed was the polyvalent live virus vaccine consisting of seven immunological types which was developed in South Africa and prepared by the Onderstepoort laboratory.

No real effort was made to study the vaccine reaction; however, two of the stud farms in Poona kept careful clinical records of their vaccinated horses and maintained daily temperature records. Febrile reactions and swelling of the temporal fossae were reported in a number of instances. Incoordination and ataxia was also noted. A debilitated 23 year old mare died on the 14th post-vaccination day and AHS virus was isolated from her spleen.

Heparinized blood specimens from some vaccinated horses in Bombay and Poona were tested intracerebrally in adult mice for the presence of virus. The study was not planned for investigation of duration and titre of viremia and was biased in that all but six of the 29 specimens were obtained from horses with significant reactions. The results of the virus isolation attempts are summarized in Table 1. Six of eight specimens up to day 19 contained virus with titres ranging from $10^{-1.2}$/0.03 ml. to $10^{-3.0}$/0.03 ml. On day 23 post-vaccination six of 11 had virus in the blood. One out of eight horses tested from day 49 post-vaccination was found to be circulating detectable amounts of virus.

The pattern of mortality in the mice inoculated with blood from vaccinated horses was quite unlike that observed in mice inoculated with blood from naturally occurring infections. The incubation period was from four to six days post-inoculation and all mice sickened within 24 to 48 hours after the incubation, or failed to sicken at all. In other words, the pattern was that of a mouse adapted strain rather than a naturally occurring one. In neutralization tests employing the convalescent serum from naturally infected horses, no inactivation of the viruses isolated from the vaccinated horses could be demonstrated. The strains from the vaccinated horses were therefore different from the epidemic strains. Sera from 67 horses obtained five and seven weeks post-vaccination were tested in NT and CF. All but one of the horses showed CF antibodies to the Jaipur strain and the titres were generally higher in the five weeks samples. Neutralizing antibody to the Jaipur strain was markedly lower than the level found in serum from horses recovered from natural infections.

Neutralization test studies at the Onderstepoort laboratory comparing the Indian strains with the Pakistan and South African strains would indicate that the Indian strains were very similar to the Pakistan strain and that the antigenic make-up of both of these differed significantly from any of the South African strains. The Indian strains were most closely related to the type 6 South African strain.

No further extensive work is planned on the African horsesickness problem at the present time save to tie up a few loose ends on projects

already in progress. A hemagglutining antigen was prepared from the Indian strain of AHS and studies on the immunological specificity of the hemagglutination inhibition reaction will be made. Many interesting problems of an epidemiological nature require further study and it is hoped that veterinarians in India will accept the challenge. Will the virus become permanently established in India in a host other than the domestic equine? What are the vector(s) species? These are two questions of paramount importance. The Indian Veterinary Research Institute, Izatnagar, has taken up the production of vaccine utilizing the strains from South Africa and have begun attempts to attenuate a local strain by prolonged mouse passage. The VRC has supplied large numbers of adult mice for the production of the vaccine and has remained ready at all times to aid any and all of those interested in the African horse-sickness problem to the limit of its resources without interfering with its other obligations.

3. KYASANUR FOREST DISEASE INVESTIGATIONS.

During 1960, 81 patients have been diagnosed positive for KFD either by isolation of virus from the blood or organs, or by serological conversion. Virus was isolated from 77 patients. In four instances, where convalescent samples were obtained, the patient failed to show a serological conversion. Four cases were diagnosed by serological conversion alone. In six cases a presumptive diagnosis was made by considering the presence of CF antibodies specifically to KFD virus in the convalescent serum as due to recent infection with that virus. In four of these cases the titre was 1/32 or greater. In one case, the titre was only 1/8 but the convalescent sample was not obtained until the 93rd PI day. The last case had a titre of 1/32 to KFD, 1/16 to JBE and no CF antibodies to West Nile virus. A number of the doubtful cases may be resolved one way or the other by NT. Convalescent samples were obtained from 47 persons. Nine deaths occurred among the cases and virus was isolated from each prior to death. Based on 87 cases, the case-fatality rate was about 10%. Only one autopsy was obtained and virus was isolated from several of the organs. The laboratory data available concerning the cases has been summarized in Table 2.

The entire surveillance program this year was carried out by members of the Mysore Health Department working through the office of the Kyasanur Forest Disease Special Officer. The isolations of virus from human cases were all done at the Virus Diagnostic Laboratory at Shimoga. Table 3 summarizes the surveillance work for the year. The number of visits made during the year was much smaller than last year. In 1959 a special effort was made in order to try and obtain data necessary to the evaluation of the vaccine. In addition, the number of personnel available for the visits was larger. In spite of this more cases were found during 1960 than any previous year. Also, the number of proved deaths was greater.

REPORT FROM DR. SCOTT B. HALSTEAD AND DR. PHILIP K. RUSSELL, WALTER REED ARMY INSTITUTE OF RESEARCH WASHINGTON, D. C.

A virus recovered from the blood of a Thai child contracting Thailand hemorrhagic fever in Bangkok in May 1960 has received considerable study in this laboratory in view of its unusual biological properties. The agent, KLA 16, produces a unique hemorrhagic disease in common laboratory rodents and additionally demonstrates marked autointerference in certain host systems. KLA 16 is inactivated by sodium desoxycholate and ether. Serologically it is a group A arthropod-borne virus closely related, if not identical, to Chikungunya virus; it is immunologically identical and biologically fairly similar to TH 35 and BaH 306, viruses recovered from Thai HF patients by Hammon and Kitaoka, respectively.

From patient's blood obtained on the 2nd day of disease, virus was recovered simultaneously in 1-2 day old mice and in trypsinized explants of hamster kidney and rhesus kidney cells. These three isolates were transmissible interchangeably in each of the three host systems, and were immunologically identical. Intracerebral inoculation of virus grown in each of these host systems produces easily recognizable intestinal hemorrhages in suckling mice, rats and hamsters. Subcutaneous, intra-articular and bladder wall hemorrhages are also seen but less frequently. The occurrence of overt hemorrhagic disease in mice is dependent upon several recognized host and virus factors. When 10 to 100 intracerebral LD_{50} of virus, obtained by passing brains from hemorrhagic mice, is inoculated intracerebrally in 30 to 36 hour-old mice, overt intestinal hemorrhage occurs with greatest frequency. Use of other routes of inoculation, of mice younger or older than the age stated, of unselected virus variants, or of larger doses of virus, reduces the frequency of hemorrhage observed.

When mice are infected under optimal circumstances normal activity decreases about 72 hours after inoculation. All or portions the small intestine become salmon pink in color and when viewed under 20X magnification, marked dilatation and congestion of blood vessels in the intestinal wall is seen. Within a few hours the color changes from pink to grey and as further bleeding occurs into the lumen, segments or large portions of the bowel turn black. Black bowel contents are strongly benzidine and guaiac positive.

The mechanism for hemorrhage is at present poorly understood, although certain defects in mouse hemostatic mechanisms have been observed. Platelet counts are sharply depressed in KLA 16 hemorrhagic

mice (ave. 220,000/mm^3) as compared with normal mice of the same age (ave. 920,000/mm^3) and with mice moribund with Chikungunya virus infection (ave. 700,000/mm^3) or with dengue 2 virus infection (ave. 930,000/mm^3). Tail bleeding times are approximately 5 times longer in KLA 16 hemorrhagic mice compared with normals and with mice infected with Chikungunya and dengue 2. Capillary tube clotting times are approximately 3 times longer than those for mice in the other groups.

Overt hemorrhage has not been observed in animals infected with TH 35 virus (examined at 18th mouse passage) and rarely in mice infected with BaH 306 (examined at 5th mouse passage). However, both of these viruses produce hematologic changes which are distinctly abnormal.

Inoculation of low dilutions (up to 1:100) of infectious mouse brain suspensions or of fluids harvested from infected hamster kidney cells into the suckling mouse results either in failure to produce death or a delay in death of mice compared with the average day of death resulting from inoculation of more dilute preparations (10-3 to 10-7) of the same specimen. Inoculation of these materials into hamster kidney tissue culture, however, does not produce autointerference at any dilution. The "interfering factor" is stable at low pH; other experiments designed to show whether or not autointerference is associated with an interferon-like substance are as yet incomplete. An interesting disassociation between the time of appearance of virus infectivity and the appearance of the "interference factor" can be shown. Mouse brain harvested at 24 to 48 hours after infection with KLA 16 virus, has high infectivity (up to 10-7.5) but produces no autointerference. But at 60 hours after infection and thereafter the "interference factor" can be demonstrated in increasing amounts.

BaH 306 infected mouse brain has similar autointerference properties; TH 35 infected mouse brain shows some autointerference but to significantly lesser degree.

Some difficulty was experienced in preparing satisfactory hemagglutinating antigens in suckling mouse brain at low passages of KLA-16 virus. However, satisfactory antigens for KLA-16 as well as TH 35 and BaH 306 can be easily prepared in hamster kidney tissue culture. Infected monolayer cultures maintained with 100% beef amnionic fluid show complete cell destruction 3 or 4 days after inoculation. Fluids harvested at this time by freeze-thawing then buffered to pH 9 with an equal volume of Borate-KCL have hemagglutinating titers up to 1:256.

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

I. JAPANESE B ENCEPHALITIS VIRUS VACCINE

A. Live, Attenuated Strains. Through a combination of up to 60 serial passages in hamster kidney tissue culture (HKTC), low temperature (24°C.) and plaque selection for low mouse intracerebral (i.c.) virulence and high HKTC titers, several strains of greatly reduced virulence have been obtained. The present pool most extensively tested and from a second plaque selection has the following characteristics: (1) HKTC $TCID_{50}$ at 24°C of $10^{8.2}$; weanling mouse i.c. virulence of $10^{1.5}$; suckling mouse subcutaneous (s.c.) virulence of zero; monkey i.c. virulence of 1/6 (1 of 2 at 10^{-1} dilution had mild encephalitis at 18 days and recovered), and in Col. Tigertt's WRAIR laboratory reported negative in Mexican burros, s.c., and monkeys s.c. This virus is considered a likely candidate for human testing in the near future.

B. Inactivated HKTC Vaccine. Hexadecylamine, though adsorbing and inactivating the virus, did not produce an immunizing vaccine. Methodology for obtaining higher titered HKTC fluids for inactivation has been improved. Titers in excess of 10^9 per ml are readily obtained in a few hours.

II. PHILIPPINE HEMORRHAGIC FEVER

A. Human Aspects. Sera from another urban outbreak have been tested, revealing dengue viruses as apparent cause. Numerous outbreaks are occurring in current years.

B. Mosquito Isolates of 1956. P-886 (Sindbis related but apparently not identical) was referred to Rockefeller Foundation Central Reference Laboratory (RFCRL) for final classification and further confirming studies by us are reported. P-581 (not groups A, B, C, Bunyamwera or California) also was referred to RFCRL over a year ago; no reported progress. Other unidentified mosquito isolates (P-422, P-759, P-830, P-870, and P-935) because of extremely low suckling mouse titers have not progressed significantly toward identification.

III. THAILAND HEMORRHAGIC FEVER

A. Bangkok Epidemic of 1960. Following a small outbreak in 1959 another major epidemic has occurred in 1960. Paired serum specimens have been received and others are enroute in large numbers from Dr. Nelson of the Bangkok Sanitarium and Hospital. Serological results on natives closely parallel those of 1958 but dengue viruses appear to play an even more significant role than chikungunya in those tested to date of 1960. There is still evidence of some dual infection. Six virus isolates from acute phase serum all behave like dengues but no identifications are complete.

B. Identification of TH-35 (chikungunya related "H" fever prototype of 1958). Vaccination cross challenge with chikungunya and TH-35 fails to detect any differences. TH-35 is not Gulu. We still consider TH-35 to be chikungunya by antigenic criteria, although the biological activity is different.

C. Identification of TH-36 (dengue II related "H" fever prototype of 1958). Vaccination cross challenge between TH-36 and dengue II NG-C strain shows no differences. Three American dengue virus infections, two in Bangkok and one in our laboratory, gave unique CF results with a battery of well-controlled dengue antigens. Titers rose strikingly to dengue IV and TH-36 but remained at $<1:4$ for dengue I, II (NG-C) and III. Thais and Chinese all rose to all dengues. We consider this specific difference shown by Americans between dengue II and TH-36 as strong evidence to support consideration of TH-36 as a new dengue type.

D. Identification of TH-SMAN (dengue I related "H" fever Bangkok virus of 1958). This virus has an antigenic pattern broader than Hawaiian but so far still appears to be a type I strain.

E. Other Isolates from Human "H" Fever Cases. Several more viruses from 1958 have been identified as similar to TH-35 or TH-36. Several which could not be readily adapted to suckling mice have been lost. A number are still in passage but unless titers increase or a more susceptible animal or T.C. system found, they cannot be used in standard serologic identification procedures.

F. Isolates from Mosquitoes. T-55 from A. aegypti is a possible dengue III, the first from Bangkok. T-185 from C. quinquefasciatus is probably chikungunya, the first from mosquitoes outside of Africa. T-172 and T-174, also from C. quinquefasciatus, are eastern equine encephalomyelitis, the first in Asia, but recognized in the Philippines.

IV. DENGUE VIRUSES IN TISSUE CULTURE

No spectacular progress.

V. BROAD GROUPING SERA FROM GROUP A VIRUSES

In varying orders WEE, EEE and chikungunya have been given subcutaneously to the same guinea pigs. These were bled and checked against group A antigens. No breadth of antibodies developed. The primary immunization appeared to prevent all subsequent infections and antibody developed only to the first virus and titers to this fell during the sequence of injections with other group A agents.

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

From cases of Japanese encephalitis reported during the period 1955-1959, age, sex, geographic distribution and seasonable incidence of this disease on Taiwan were determined. The season is short, 3-6 weeks, and occurred as early as June and as late as September. The seasonal pattern was confirmed by specific Japanese encephalitis serological tests on sera from patients in 1958 and 1959. The virus of Japanese encephalitis was also recovered on Taiwan for the first time. Tests on sera of American servicemen collected before and after the 1959 season showed that 5% of personnel on Taiwan and 3% on Okinawa had developed antibodies (and had been presumptively infected). Collaborative studies with the Dept. of Entomology on the severe epidemic of 1960 are in progress.

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

Forty-three presumptive Japanese encephalitis (JE) virus isolations were made from 651 pools of mosquitoes collected on Okinawa by the Entomology Section, U.S. Army Medical Service Group under the direction of Major Carlyle Nibley, Jr., and shipped to Taipei by air. The mosquitoes were preserved on dry ice until tested, usually within 2 weeks of the day of collection. The largest number of isolations was made from the September collections when Culex tritaeniorhynchus was most numerous and 20-30% of the pools of this mosquito were positive. Most of the mosquitoes and isolations were from the village of Geoku near the center of the island of Okinawa where sampling was started in March and continued until the middle of November.

Six virus isolations were obtained from 198 pools of mosquitoes collected near Taipei, Taiwan, during August, September, and October. Five of these are probably JE virus, 3 from C. tritaeniorhynchus

and 2 from Culex fatigans. Three presumptive JE virus isolations were made from sentinel suckling mice exposed in the Taipei area during the month of September.

A serological survey in 1959 indicated that about 4 per cent of marines converted from negative to positive for JE during the first season of residence on Okinawa, a second group is being tested but this work is not yet completed.

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

Adsorption of West Nile virus to chick embryo monolayers subsequently to form plaques under agar is pH dependent, yielding a biphasic curve with maxima between pH 5-6 and pH 8-9 and a minimum at about pH 7. Adsorption rate is high at the acid pH and low at the alkaline pH; thermal inactivation rate is high at the acid pH and low at the alkaline pH. The biphasic curve can probably be explained in large part by the interaction of these two processes.

Four potentially attenuated type 1 dengue strains were tested in human volunteers. Three produced mild systemic reaction, rash and neutralizing antibodies. One produced no symptoms or rash but only 2/4 volunteers developed neutralizing antibodies with the dose employed (4.2 logs suckling mouse ID_{50}). Dissociation between HAI and neutralizing antibodies was noted in several instances.

A serological survey of sera from indigenous populations of the Cape York Peninsula of Australia, New Guinea, New Britain and other Pacific Islands has been undertaken. Results with HAI tests employing 2 group A (Semliki, Chikungunya), 7 group B and 3 group C antigens are now coming available. In Cape York, two different patterns were observed. The group A antigens gave positive results in 52% of the sera with positives distributed essentially equally in all age groups from less than 1 year to adults. Dominence patterns of group A antibodies in different localities suggest the possible occurrence of more than one group A virus. With group B antigens, there was a progressive increase in % positives until about the 12 year age group when essentially all persons had group B antibodies. Of the antigens tested, MVE gave the dominant pattern. Little or no evidence was gained for the presence of dengue virus (1-4) infections. Antibodies against group C viruses were absent. Results with sera from New Guinea are not yet nearly complete. However, the antibody pattern appears to be much more complex. No group C antibodies were detected. Group A and B antibodies

are very prevalent in lowland areas. In highland areas, the incidence of group B antibodies is very low but group A antibodies continue to occur in appreciable numbers.

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

In the last report it was noted that there had been one case of Western equine encephalitis in California in 1960. There were no further cases of Western equine encephalitis. There were 12 cases of St. Louis encephalitis with onset between July 14 and September 27, 1960. These cases were from Sutter, Sacramento, Colusa, Madera, Fresno, Tulare and Imperial Counties. One case was listed as from the Central Valley because the exposure could have occurred in more than one county. There were 4 cases in Fresno County between September 10 and 16. An additional case of St. Louis encephalitis was identified in a patient exposed when visiting in Loveland, Colorado. The date of onset of this case was August 22.

There were 6 proved cases of Colorado tick fever contracted in California in 1960. These were from Modoc, Lassen, Plumas, and El Dorado Counties. The dates of onset were from April 13 to June 22. An additional case was identified where the exposure had occurred in Colorado. The date of onset of this case was April 22.

The 1960 field studies of small mammals of the Great Basin Plateau yielded 7 isolations of Colorado tick fever virus from blood specimens obtained from four different species of mammals. Three of these were described in the last report. In addition the blood specimens from 3 yellow pine chipmunks, Eutamias amoenus, trapped September 7 and 8, and a wood rat, Neotoma fuscipes, trapped September 7, yielded Colorado tick fever virus. The virus strains isolated from Dermacentor andersoni ticks all proved to be Colorado tick fever virus. Two were from single tick specimens collected at sites where the virus had been isolated from small mammals. The other four isolations were from pools of ticks collected on Sugar Loaf Lookout Mountain at an elevation of 7312 feet.

An attempt was made to infect Culex tarsalis mosquitoes with the Kern Canyon virus isolated from Myotis yumanensis bats. Mosquitoes fed on infected mouse brain were negative for virus when tested three and four weeks later.

Cross immunity studies in mice showed that Powassan virus was the same as the virus isolated by Dr. Carl M. Eklund, from Dermacentor andersoni ticks collected in Colorado in 1952. *

By selection of clones by the plaque technique it has been possible to obtain a clone of Western equine virus which produces an asymptomatic infection in young adult mice inoculated intracerebrally. The mice immunized with this cloned virus are immune to intracerebral challenge with neuroadapted strains of Western equine virus.

*Thomas, L. A., Kennedy, R. C., and Eklund, C. M., Isolation of a virus closely related to Powassan virus from Dermacentor andersoni collected in Cache la Poudre Canyon, Colorado, Proc. Soc. Exp. Biol. & Med., 104:355-359, 1960.

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

This report reviews highlights of accomplishments since April 1, 1960, the closing date of the last Annual Report on "The Ecology and Control of Arthropod-borne Viruses in Kern County, California". (This is a cooperative project of the University of California, the Encephalitis Section of the Communicable Disease Center, and the Bureau of Vector Control, California State Department of Health). An annual report will be released in May, 1961, and the present review will be brief.

Analyses are being made of the first year's efforts to intensively control Culex tarsalis in an 18 square mile area as a means of determining if WEE and SLE virus transmission can be interrupted in a highly endemic area. An intensive larval control program in 1960 was at least 90% effective in preventing the emergence of adult C. tarsalis within the 18 square mile area. However, as the summer progressed there was increasing evidence of a substantial infiltration of female C. tarsalis into this area from breeding sources outside of the area of control. Light trap, bait trap, and red box measures of adult C. tarsalis populations still indicated a material reduction of population as compared with a nearby comparison area where C. tarsalis control was minimal. Both WEE and SLE viruses were recovered and were transmitted in the control and comparison areas, but the levels of virus activity were universally low in all of Kern County in 1960. It is concluded that the control program failed to reduce the C. tarsalis population to a threshold level, where virus transmission would be interrupted. The possible relationship of infiltrating female C. tarsalis, possibly virus infected, to virus activity in the control area must still be clarified. This will require extensive flight range studies and an intensive search for the source of infiltrating adults in the summer of 1961. The intensive control program and evaluation of virus activity will be continued in 1961 but the area of control will be enlarged from 18 to 27 square miles.

A long-term study has been established to determine the duration of antibodies to WEE and SLE viruses in persons who have had inapparent infections. Blood samples were obtained on 261 volunteers in July, 1960. Repeat bloods were obtained on 177 of these volunteers in October, 1960. In HAI tests on the first blood samples, 40 had WEE antibodies and 84 had SLE antibodies. The rate of antibody loss and accretion will be followed in this population. In a previous study of residents of this highly endemic area, 9 of 12 WEE positives and 3 of 19 SLE positives converted to negative within an 8-year period.

There is increasing evidence that Powassan virus or a very closely related virus is active in Kern County. Two rodents have been collected that had HAI titers above 1:20 to this agent and that had no detectable HAI antibodies to SLE, Modoc, Rio Bravo, or Jap B virus. Virus isolations will be attempted from blood samples and ectoparasites collected from rodents this spring.

Dr. Ian Marshall is completing studies on the characteristics of WEE virus plaques on monolayers of chick embryo fibroblasts. Interesting variations have been found. Freshly isolated field strains from mosquitoes and birds uniformly produce large plaques (ave. 9 mm diameter). This characteristic is maintained on passage through embryonated eggs, and, less reliably, on mouse brain passage. Passage through chick embryo fibroblast or hamster kidney tissue cultures results in a rapid change to small plaques (ave. 2 mm diameter). This plaque size marker offers interesting avenues for future studies of variations in pathogenicity and antigenicity.

Laboratory studies are nearly completed on samples collected at Hermosillo, Mexico, in April, 1960. HAI serological tests revealed the following distribution of antibodies in various hosts:

WEE - horse (unvaccinated) and man
EEE - chicken
SLE - horse, man, and rodent
Powassan - chicken and rodent
Dengue I and II - man

As bloods from all hosts were tested with all the above antigens (except Dengue for hosts other than man), as well as with three additional Group B antigens, the selective distribution of antibodies in the various hosts poses several interesting epidemiological and immunological problems. Current interpretation is that all six viruses may have been active in this area of Mexico as one or more hosts were found with antibodies to only one of the above agents. Unfortunately, the

limited field collecting period during the early spring did not result in isolation of any of these viruses. One Rio Bravo virus isolation was obtained from a bat by Doctor H. N. Johnson.

REPORT FROM DR. J. V. IRONS, DIRECTOR OF LABORATORIES TEXAS STATE DEPARTMENT OF HEALTH, AUSTIN, TEXAS

Summary on "Arbor Virus" Studies in Texas in 1960

Laboratory confirmations based on complement fixation tests were obtained on 17 cases of WE and 2 cases of SLE in 1960. Cases were widely scattered but mainly occurred in the Plainview-Lubbock area of the West Texas High Plains where irrigation is practiced. One of the SLE cases, that of a 65-year-old female, expired. One laboratory-acquired infection (WE) occurred in the state.

Three of the four laboratory confirmed equine cases occurred in the West Texas High Plains. The fourth case was in Cameron County in the lower Rio Grande Valley. No proved Eastern equine case has been encountered in several years.

Approximately 10,000 mosquitoes, principally culicine, collected in the El Paso, Texas-Juarez, Mexico, lower Rio Grande Valley and Lubbock-Plainview areas, were tested for virus content. The WE virus was recovered only from 8 pools involving 349 of the 2451 Culex tarsalis collected in the Lubbock-Plainview area. The SLE virus was recovered from 9 of 26 pools of C. quinquefasciatus and 4 of 36 pools of C. tarsalis collected in the Texas High Plains in 1959.

In the Texas High Plains HAI tests of sentinel flocks of chickens showed considerable WE and SLE virus activity both in 1959 and 1960. SLE virus was found in tissues of a few white wing doves in Cameron County in 1959, but no virus was found in tissues of birds in 1960.

The finding of SLE virus in a pool of C. quinquefasciatus collected in Cameron County in January 1958, suggested possibly that the virus was over-wintering in this mosquito. However, none of the several pools of mosquitoes subsequently collected in the Lower Rio Grande Valley have yielded SLE virus.

In 1958, a small pool of Anopheles pseudopunctipennis yielded a virus, #20230. This virus shows some of the properties of the "California" virus. Quite recent serological and protection tests indicate that #20230 and "California" virus definitely are related.

During the present season more extensive testing of pools of mosquitoes including non-culicine from additional areas is planned. More attention is to be directed toward the role of birds and small mammals in the basic infection chains. More adequate follow-up of undiagnosed cases of summer encephalitis, particularly by enlarging upon the "battery" of serological tests is planned. Many cases of "encephalitis" turn out to be polio or aseptic meningitis, on basis of serological or cell culture tests; however, several cases of "encephalitis" remain undiagnosed each season.

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

Correlations Between Temperatures and Encephalitis Transmission Rates

Since the time of the last report, extensive analyses have been made of the relationships between temperatures in the Greeley area during the past seven years and WE and SLE transmission rates as measured by HAI antibody rates in avian sentinel flocks. These studies have revealed that there is a close positive correlation between spring temperatures and SLE transmission rates; for WE, however, there is a negative correlation between spring temperatures and transmission rates.

During the 1960 season, antibody data were obtained from standard sentinel and farm chicken flocks at different elevations in Colorado. The results were in agreement with the seven-year study in that there was no transmission of SLE at the higher, colder elevations, whereas maximum transmission rates for WE occurred as high as 8,500 feet. Transmission of WE at some of the higher elevations occurred under conditions that make it highly doubtful that Culex tarsalis could have served as the vector.

Transmission rates of WE and SLE in avian sentinels at various sites in the western states also are in agreement with the above temperature relationships. SLE activity is consistently low at the more northern, colder latitudes, whereas maximum levels of WE activity occur all the way to the Canadian border. Finally, the distribution of past human outbreaks of WE and SLE suggest a similar relationship. SLE outbreaks have been fairly well limited to the area south of a line marking the 70°F isotherm for the month of June. Outbreaks of WE, however, occurred through the northern states and into Canada.

Observations on the Nulliparity of Culex tarsalis

Serologic studies with avian sentinels have shown no correlation between population densities of Culex tarsalis and transmission rates for WE and SLE. In an attempt to find an explanation for this situation, the parous or nulliparous condition of C. tarsalis was studied during the 1960 season in areas of high and low mosquito population densities. The results show that the per cent of parous mosquitoes in the area of low densities was only slightly higher than that found in specimens from areas with a high mosquito population density. This slight difference in nulliparity rates probably was not great enough to account for the difference in virus transmission. In areas where the populations of C. tarsalis were large, specimens collected in CO_2 can traps showed a progressively increasing percentage of parous individuals through late summer and fall. In contrast, mosquitoes collected from resting stations showed a progressively increasing percentage of nulliparous individuals as the season approached fall. These data would tend to indicate that blood feeding mosquitoes have a tendency to continue this activity and egg laying until all of the declining population is parous. Those mosquitoes found in daytime resting shelters constitute a population less interested in blood feeding as the season progresses and perhaps represent a prehibernation population.

Nulliparity studies also were made on 754 C. tarsalis hibernating in abandoned mine tunnels. Of the mosquitoes examined, only two were parous. Since almost all of the hibernating mosquitoes found in the mines were nulliparous, it appears highly unlikely that C. tarsalis serves as a primary overwinter reservoir of encephalitis viruses at this latitude. Additional nulliparity studies on C. tarsalis emerging from hibernation are presently in progress.

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

COLORADO TICK FEVER

Isolations from Colorado Tick Fever Patients - 1960*

State	April	May	June	July	August	Unknown	Total
California			1				1
Colorado	2	1	4				7
Idaho		6	4	1		2	13
Montana		1		1	1		3
Nevada	2		1				3

Continued

Continued

State	April	May	June	July	August	Unknown	Total
Oregon	2	3	4	2			11
So. Dakota		1					1
Utah			1				1
Wyoming		1	4	3		1	9
Totals	6	13	19	7	1	3	49

*One 9-year-old boy from Oregon had definite symptoms of encephalitis with increased cell count in the spinal fluid and a petechial eruption on arms and shoulders.

Dermacentor andersoni - 1960

A total of 1890 ticks were examined from Colorado, Wyoming, and Montana, without adding Colorado tick fever immune serum and 91 strains of Colorado tick fever virus were isolated. A total of 2619 were examined with immune Colorado tick fever serum added - no isolations of the Powassan virus were made from either group.

Isolation of Virus from Culex tarsalis - 1960

State	No. Mosq.	No. Pools	+WEE	+SLE
Idaho	2248	112	15	6
No. Dakota	5374	267	2	0
Oregon	1337 (majority collected 8/7-8/13)	58	10	1
Washington	1178	40	1	0
Totals	10,137	477	28	7

Culex tarsalis are being followed through the winter in Vale, Oregon in rock piles. Over 600 Culex tarsalis were collected from rock piles near Vale, Oregon from November 29 to December 4 and from January 11 to 16. The lowest daytime temperature observed during January was 24°F and highest was 49°F. Within the rock piles an inversion of temperature was noted--temperatures ranging from 24.5°F to 29°F. At 26°F, C. tarsalis were able to walk slowly and at 37°F were able to fly about 2 feet. At 40° they flew about 50 feet. Similar observations were made regarding C. incidens and A. freeborni.

Isolation of virus from Culiseta inornata - 1960*

A total of 4658 Culiseta inornata ground in 243 pools from North Dakota yielded 6 isolations.

*Four isolates of this virus were made in 1952 from C. inornata. This virus is related to Cache Valley virus and Bunyamwera virus. From the latter it can be separated by cross neutralization tests although closely related by the HI and CF tests.

Wisconsin workers reported the presence of EEE antibodies and the isolation of EEE virus from turkeys in Wisconsin. Because of this, hog and turkey sera from North Dakota have been examined. We have found no EEE antibodies to date but with some hog sera there is evidence of slight neutralization which we believe to be nonspecific.

In connection with his work on the biology of C. tarsalis in Washington, Dr. Harwood, of the Washington State University at Pullman, has found Phlebotomus in eastern Washington--extending the known range of this species of fly in the U. S. far to the north.

REPORT FROM DR. WILLIAM F. SCHERER
DEPARTMENT OF BACTERIOLOGY
UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
MINNEAPOLIS, MINNESOTA

Investigations of arthropod-borne viruses in cell and tissue cultures have extended studies of the mechanism of overt JE viral cytonecrosis of chicken embryonic cells under agar medium and its absence in fluid medium. Actually a few cells in cultures with fluid medium are destroyed, quickly detach from glass, and empty spaces are promptly covered partially or completely by migrating cells which in turn are protected from the destructive effects of virus by an interfering substance produced in infected cultures. With solid medium, cell destruction is recognized because healthy cells cannot migrate, and there is limited flow of interfering substance throughout the cultural environment. Whether less interfering substance is produced by cells under solid medium than by cells under a liquid medium has yet to be determined. Virus titers are essentially equal in suckling mice (LD_{50}) and in chicken embryonic cell cultures (PFU) when mice receive 0.02 ml and cultures 0.2 ml of inoculum.

Cells from a continuous porcine kidney cultures, like those in primary porcine kidney cultures, are destroyed by JE virus in cultures containing fluid medium; under agar, tiny plaques occur.

Sagiyama virus passes 100 but not 50 millimicron filters, causes lesions in suckling mice that resemble those of group B Coxsackie viruses, and produces viremia, but no overt disease in swine inoculated subcutaneously. One strain of Sagiyama virus (M7/504) isolated from mosquitoes collected in 1957 and used in first mouse passage for inoculation of pigs produced the usual HI, N, and CF antibody response; the prototype strain (M6/Mag 132) in 13th mouse passage produced neutralizing but no HI or CF antibody responses. Moreover, the neutralizing substance, though evidently specific for Sagiyama virus, was heat labile. Ecologic studies of swine infection by Sagiyama virus during 1956 and 1957 reveal a high frequency (70%) of infection at the Sagiyama and Shinhama heronry areas north and east of Tokyo and the Zama area south of Tokyo. Most swine infections occurred during the late July - early September interval in 1956-57, and in individual pigs, were often unrelated temporally to JE virus infection.

Mag 115 virus (an unclassified virus recovered from mosquitoes in Japan during 1956, resistant to sodium deoxycholate and ether, though able to propagate serially in inoculated arthropods) passes 100 and probably 50 millimicron filters. This virus is unrelated by complement fixation and neutralization tests to any known enterovirus, to a variety of arthropod-borne and miscellaneous viruses, and to Akabane virus, a new virus isolated from mosquitoes by Dr. M. Kitaoka and colleagues at the Japanese N. I. H. It is pathogenic for suckling mice intraperitoneally as well as intracranially. Maximum virus yields are obtained from mouse muscle; limb and paravertebral muscles show severe myositis and degeneration, brown fat is focally necrotic and spinal cord neurons are damaged. Preliminary survey for neutralizing antibody in sera of man, pigs, and birds in Japan reveals to date no antibody in humans, unequivocal neutralizing antibody in pigs bled in 1956 and 1957, and antibody in one Black-crowned Night Heron. In July 1960, Mag 115 virus was sent to Drs. Theiler and Casals for study.

An unclassified virus (M7/270) recovered from "Anopheles sinensis" collected at Sagiyama, Japan, in 1957, remains unclassified and difficult to work with because suspensions of infected brain or other tissues yield low titers of infectious virus. Currently serial passages are being continued in attempts to adapt the virus further to suckling mice.

An unclassified virus (Tsuruse) recovered from blood of a Blue Magpie (271580) near Tokyo in 1954 passes through 100 millimicron filters, is inactivated by sodium deoxycholate and diethyl ether, is pathogenic for both weanling and suckling mice inoculated intracranially, kills chicken embryos inoculated by yolk sac, and produces lesions on the chorioallantoic membrane but no agglutinins for goose erythrocytes in allantoic fluid. It produces plaques in chicken embryonic cell cultures with agar-medium, but no cytodestruction in cultures of primary human amnion or rhesus monkey kidney cells in fluid-medium. It is not

neutralized by JE, Sagiyama, Mag 115 or M7/270 virus antisera and does not react in complement fixation tests with psittacosis, Sagiyama, Mag 115, JE, Sindbis, mumps or herpes simplex virus antisera. Previous tests at WRAIR failed to show neutralization with rabbit antisera specific for JE, Ntaya, Zika, Ilheus, Uganda, Bunyamwera, Wyeomyia, West Nile, SLE, MVE, yellow fever, dengue types 1 and 2, Semliki Forest, Sindbis, Mayaro, GDVII, encephalomyocarditis, lymphocytic choriomeningitis, MM1775, Bat (1410-1419) and RSSE SpN4.

Another unclassified virus (BCNH K622) recovered from a Black-crowned Night Heron near Tokyo in 1954 is resistant to sodium deoxycholate and diethyl ether, kills suckling and weanling mice inoculated intracranially, and does not kill primary human amnion cells, primary rhesus monkey kidney cells or HeLa cells. It produces ulcerated thickened lesions on the chorioallantoic membrane of embryonated chicken eggs, but does not kill embryos after yolk sac inoculation or produce goose erythrocyte agglutinins in allantoic fluid. It is not neutralized by antiserum specific for JE, Sagiyama, Mag 115 or Tsuruse viruses.

Studies have continued of capturing technic of birds (shooting vs. netting) as a factor in surveys for JE viral neutralizing antibody. Chicks passively immunized either by subcutaneous inoculation of homologous JE viral antibody or by hatching from immune hens can develop detectable neutralizing substances in plasma obtained by cardiac puncture immediately after shooting, despite the fact that neutralizing substances are undetectable in plasma obtained by jugular venipuncture before shooting. In certain instances these substances appear to be nonspecific since they also neutralize Western encephalitis virus. However, when wild birds from Minnesota are examined, some show neutralizing substances to WE virus in after-shooting plasmas (but not before-shooting plasmas) which specifically neutralize WE and not JE or SLE viruses suggesting that shooting can also engender detectable levels of specific antibody in plasma. These latter findings are consistent with previous observations of after-shooting plasmas from Japanese birds where the presence of HI as well as neutralizing substances provides strong evidence that the substances are antibodies.

ON ETHER AND SODIUM DESOXYCHOLATE INACTIVATION OF VIRUSES

December 1960

Viral infectivity unchanged

Size range (proved or probable)	DNA RNA	Diethyl ether	Sodium deoxycholate
120-200 mμ	D	vaccinia (1)	vaccinia (8, 9)
	D	ectromelia (1)	ectromelia (8, 9)
		goat pox (5)	
		?sheep pox (5)	
		(?fowl pox)	
		?rabies (4)	
			psittacosis (9)
50-120 mμ	D	adenovirus 1, 2, 3 (11)	adenovirus 1, 2, 3 (11)
	R	(?vesicular exanthema)	
15-50 mμ		bacteriophage staph K (1)	
	D	bacteriophage coli 36 (1)	
		bacteriophage shigella 13 (1)	
	R	poliovirus (4, 7, 11)	poliovirus (8, 10, 11)
	R	Coxsackie A-9, B-1 (11)	Coxsackie ?type (10); A-9 & B-1 (11)
	R	Echo 1, 6, 9 (11)	Echo 1, 6, 9 (11)
	R		encephalomyocarditis-Mango (10)
	R	foot & mouth disease (1)	foot & mouth disease (8)
		mouse encephalomyelitis-FA(1)	mouse encephalomyelitis-FA & GDVII (10)
	D	rabbit papilloma (1)	
	D	polyoma	

December 1960

Viral infectivity reduced >1 log

Size range (proved or probable)	DNA RNA	Diethyl ether	Sodium deoxycholate
175-330 mμ		feline pneumonitis (1)	
		lymphogranuloma venereum (3)	
		mouse pneumonitis (1)	
		psittacosis (2)	
		trachoma (13)	trachoma (13)
120-200 mμ		B virus (1)	
	D		pseudorabies (9)
	D	herpes simplex (1)	herpes simplex (9)
		grey lung virus of mice (1)	
	D	rabbit myxoma (1)	rabbit myxoma (9)
	D	rabbit fibroma (1)	
		?sheep pox (12)	
		rabies (15, ?6)	rabies (8)
		measles (15)	
		rinderpest (15)	
		canine distemper (15)	
50-120 mμ		bunyamwera (11)	bunyamwera (10, 11)
			wyeomyia (10)
			anopheles A (10)
			anopheles B (10)
			Ntaya (10)
			bwamba (10)
	R	influenza A & B (1, 11)	influenza A (8, 9, 11), B (11)
		mumps (1)	
		Newcastle disease (1)	Newcastle disease (9)
		fowl plague (1)	
	R	Rous sarcoma (1)	Rous sarcoma (8)
	R	(?vesicular stomatitis)	
15-50 mμ		lymphocytic choriomeningitis (1)	
	R	eastern encephalitis (4)	eastern encephalitis (10, 11)
	R	western encephalitis (4)	western encephalitis (10)
		Sindbis (11)	

December 1960

Viral infectivity reduced > 1 log

Size range (proved or probable)	DNA RNA	Diethyl ether	Sodium deoxycholate
	R	Semliki forest (11)	Semliki forest (10, 11)
		Sagiyama (14)	Sagiyama (14)
			Venezuelan encephalitis (10)
			dengue 1 (10)
			dengue 2 (10)
		Ilheus (11)	Ilheus (10, 11)
	R	Japanese encephalitis (11)	Japanese encephalitis (10, 11)
		louping ill (1)	louping ill (8, 9)
			RSSE (10)
		St. Louis encephalitis (4)	St. Louis encephalitis (10)
			Uganda S (10)
	R	West Nile (11)	West Nile (10, 11)
		yellow fever (1)	yellow fever (10)
			Zika (10)
		Marituba (11)	Marituba (11)
		Oriboca (11)	Oriboca (11)
		California (11)	California (10, 11)
		Rift valley fever (1)	Rift valley fever (10)
			Sandfly fever - Naples (10)
			Sandfly fever - Sicilian (10)

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REPORT FROM MR. CLARENCE J. GIBBS, JR., ACTING HEAD ARTHROPOD-BORNE VIRUS SECTION, LABORATORY OF TROPICAL VIROLOGY, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, NATIONAL INSTITUTES OF HEALTH BETHESDA, MARYLAND

During the past year, the Arthropod-Borne Virus Section, Laboratory of Tropical Virology, issued Technical Report Number 1 - a summary of two years' work in this field of endeavor. Since that time the Section's activities have been influenced by the resignation of Dr. William L. Pond and Dr. Herbert T. Dalmat. There has been an almost complete re-staffing of technical, non-professional, personnel.

During 1960 the staff of ABVS pursued the projects initiated during the preceding calendar year. New projects were initiated that included (1) an application of the technique of antiserum pool combination to typing of ARBOR viruses, (2) a detailed evaluation of experimentally produced EEE virus infections in horses and (3) attempts to develop an antigenically potent and stable inactivated EEE virus vaccine for human use.

The summaries herewith presented represent major findings of approved projects.

I. Studies on Arthropod-Borne Viruses in the Sub-Tropical Areas of the United States.

The testing of sera from human beings residing in the Southern United States has been continued. Neutralization and hemagglutination inhibition tests performed on 125 sera from Miami residents indicate activity of SLE, EMC, and possibly Ilheus viruses. There are strong indications that another group "B" virus is active or has been active in the Miami area.

II. Studies on Antigen-Antibody Reactions of Arthropod-Borne Viruses. Kinetic Studies of the Serum Neutralization of the Arthropod Borne Viruses.

The concept of instantaneous neutralization of viruses with homologous antibody was tested using group B moderately reactive sera obtained from residents of Guatemala and group B broadly reactive sera from residents of Southeast Asia. The data obtained show that the neutralization of virus by homologous antibody is an instantaneous phenomenon. The Guatemalan sera, due to moderate cross reactivity noted in the conventional type NT, (incubation serum-virus mixtures) were easily differentiated by the neutralization indices obtained by the modified NT (no incubation). The Southeast Asian sera remained broadly cross reactive although to a lesser degree in the modified NT than in the conventional NT.

III. Studies on Antigen-Antibody Reactions of Arthropod-Borne Viruses. Development of a Practical and Specific Flocculation Test for the Demonstration of Arthropod-Borne Virus Antibodies.

Studies on the development of a rapid flocculation test employing Bentonite for the detection of arthropod-borne virus antibodies have continued. When EEE and SLE viruses were used as prototypes against homologous antisera, specific high titered reactions were noted. No cross reactivity was demonstrable in flocculation tests with related group A sera or related group B sera. However, antibody titers obtained when the same immune sera lot but different lots of infective HKTC fluids were employed, have been variable. This variability may be a function of virus antigen concentration and/or the amount of adsorption of virus to the Bentonite clay particles.

IV. Typing of Viruses by Combinations of Antiserum Pools. Application to Typing of Arthropod-Borne Viruses.

Studies were initiated designed to develop methods by which individual tests of an unknown ARBOR virus against a number of typing sera can be replaced by tests against a small number of pools of these sera. Viruses thus far employed in these studies are prototype strains of representative viruses of groups A, B, and C. These prototype viruses were neutralized to a greater extent when tested against their homologous antiserum alone or against pools which contained such antiserum. Heterologous neutralization of prototype viruses occurred only between members of the same serological group. There was no serological crossing over between groups A, B, or C. An unknown viral isolate, suspected of belonging to group C, when tested against combination serum pools was neutralized by combination of group C antisera but not by pools of groups A or B. The data thus far collected show that this method can be applied successfully in group typing of an unknown virus. These studies are being expanded to include other ARBOR virus groups.

V. A Qualitative Evaluation of Experimentally Induced Eastern Equine Encephalomyelitis (EEE) Virus Infection in Horses.

An immunological investigation of experimentally induced EEE virus infection in horses designed to elicit the formation, development and persistence of viremia and HAI, NT, and CF antibodies was undertaken in collaboration with Dr. R. J. Byrne of the University of Maryland and Major E. Buescher of the WRAIR. Results obtained show that following the exposure of horses to EEE virus, viremia is detectable within 24 hours and persists at a significant level for 72 hours. The 1 day old chick is the most efficient system for the detection of EEE virus in the horse blood. Significant levels of HAI antibodies appear on the 5th day after

inoculation, reach a high plateau between the 10th and 15th days, at which time a depression is noted. Another slight rise is noted between days 30 and 40 and this level seems to remain for at least a year. CF antibodies are first detectable between days 4 and 10 when a significant level is reached (1:4, 1:8, 1:4). By the 15th day a marked rise in CF titer is noted. The level of antibody remains high through the 50th day (1:1024, 1:2048, 1:1024) and significant levels persist through 365 days (1:32, 1:32, 1:32). NT antibodies are detectable as early as 10 days (1:3, 2:0, 1.7) after inoculation and closely follow the HAI pattern. A high NT index is noted between the 21st and 30th days (2.5, 3.8, 3.0) which persists through the 204th day (3.4, 3.6, 2.7). Subsequent bleedings have not as yet been tested.

VI. The Development of an Inactivated Vaccine Against EEE Virus.

Two lots of EEE-HKC formalin inactivated vaccine have been prepared and assayed. Our data show that neutral formalin, in the concentration employed, is capable of completely inactivating detectable viable virus without destroying the antigenicity of the product. Vaccinated animals have been able to survive IC challenge with as many as 1000 IC guinea pig LD_{50} doses of virus. Serological data show the vaccine to be capable of eliciting NT antibodies of from 1:5 to 2:2 logs. CF and HAI antibody responses have not been determined.

VII. Studies of Arthropod-Borne Viruses in Tissue Culture. Evaluation of Tissue Culture Systems for Use of Viral Isolation, Identification, and in Serological Tests for Viral Antibodies.

Hamster kidney cell cultures (HKTC) have been found to be particularly useful in propagating ARBOR viruses. Presently, more than 20 of these viruses have been grown and observed to produce cytopathic changes in this type cell line (Rosenberger and Shaw. Proc. Soc. Exp. Biol. and Med., 106: 1: 223: 1961).

A contaminating non-viral organism was isolated from apparently normal HKTC. This organism appears to be a bacterial L form. It produces a hemonuclear adsorption reaction in cell cultures which results in dissolution of the cytoplasm of nucleated chick erythrocytes leaving nuclei adsorbed onto cells of infected cultures. The organism will propagate in a suspension of chick erythrocytes in BSS. The hemonuclear adsorption phenomenon has proven to be reliable and is employed routinely in our laboratory as a test to detect the presence of this organism in cell cultures.

In collaboration with Dr. Walter L. Newton, Laboratory of Germ Free Animal Research, many cell lots have been prepared from germ-free animals. Comparative tests, with kidney cell cultures prepared from germfree and conventional mice, have not shown differences in growth or cytopathogenicity with the following viruses: Yellow Fever (French neurotropic strain), Anopheles A, Murray Valley Encephalitis, and Oriboca. Previously reported preliminary results of greater cytopathogenicity with Dengue type 1 virus (Mochizuki strain) in kidney cells prepared from germ-free mice than in cells from conventional mice have not been consistent on additional investigation.

VIII. Studies of Arthropod-Borne Viruses in Tissue Culture. Development of a Cell Culture System Utilizing Arthropod Tissue.

Studies into the development of methods of preparing cell cultures from arthropod tissues have been continued. Treatment of silkworm ovarian tissue with a balanced salt solution extract of the crop of the blue crab causes disassociation of cells. This treatment provides a suspension of cells rather than tissue fragments or clumps of cells. In culture, cells attach to the glass substratum but fail to proliferate in Wyatt's medium when held at a variety of temperatures of incubation.

IX. Survival Potential of the Adult of Haemagogus Equinus, A Sylvan Vector of Yellow Fever.

A study of the survival potential of the adult of Haemagogus Equinus, a sylvan vector of yellow fever, is being carried out. The survival of H. equinus adults is greatest in those populations that arise from eggs laid by young females fertilized by young males, from surviving eggs of batches that have been held for periods of time up to near the maximum periods of survival under adverse conditions, and from larvae that developed at lower temperatures, and is greatest in those populations of adults that are maintained in varied rather than constant temperatures and humidities. Survival is reduced by higher temperatures, by excessive activity induced by high intensities of white light, and by air-borne vaporized oil of exceedingly low concentration.

Under present laboratory conditions, the 70% survival point for H. equinus adults is 50 days for males and 64 days for females; the 90% survival point is 33 days for males and 44 days for females. The maximum life span has been increased from 11 days for males and 39 days for females to 107 days for males and 108 days for females. Known circumstances indicate that the survival potentials and potential life spans are still above these values.

ANNOUNCEMENT OF NEW REGISTRATION FORMS FOR ARTHROPOD-BORNE VIRUS CATALOG

More than 60 viruses have now been submitted for registration in the catalog but further registration has been held up temporarily pending issuance of a new registration form or questionnaire. When the original form was distributed it was anticipated that as a result of experience some alterations would be indicated. Indeed, in the letter accompanying its initial distribution, suggestions on useful changes were solicited.

As a result of a number of helpful and constructive suggestions, particularly from those who have sent in records on viruses for registration, and as a result of opinions expressed by members of the WHO Study Group which met in Geneva last September when the catalog was discussed, and from experience gained from reviewing the filled-in forms which the sub-committee responsible for assembling the catalog has received, it was decided to prepare a new form before proceeding further with the registration.

The first question which arose was whether the form should be abbreviated and reduced to two or possibly one page or to remain in its present four-page form. It was of interest to find that the WHO Study Group advocated more rather than less information be requested, particularly as concerned epidemiological and ecological features. This opinion was also concurred in by members of the sub-committee as well as a majority of other interested persons consulted.

Consequently, a sample form was devised covering four pages, the same as the initial one, but with some additions, rewording and rearrangement of the questions asked, and with the innovation of printing on the back of each page explanations and suggestions which it is hoped will serve as helpful guides in answering the questions and in supplying comparable data. The sample form was circulated among the "Gould House" group and to those who had submitted viruses for registration for comments and suggestions. The final printed form, which is herewith inserted, incorporates most of the suggestions offered and it is hoped may be found generally acceptable.

Anyone having a virus which may meet the qualifications for registration and wishes to submit a record for inclusion in the catalog may obtain blank forms by writing to the Chairman of the sub-committee on Exchange of Information, Dr. Richard M. Taylor, California State Department of Public Health, Viral and Rickettsial Disease Laboratory, 2002 Acton Street, Berkeley 2, California.

I. Virus name and/or number .....
Information from ..... Date .....
Address .....

II. Antigenic: Group ..... Ungrouped ..... (give details under VI C)

III. Original source:

A. Isolated by ..... at .....

B. Genus and species .....

(If from sentinel animal so indicate)

Age or stage ..... Sex .....

Isolated from: Whole blood ..... Clot..... Serum or plasma ..... Other fluids .....

Organs and tissues .....

Signs and symptoms of illness .....

.....

C. Time of collection: hour ..... day ..... month ..... year .....

D. Method of collection .....

E. Location of source when collected .....

Geographic .....

Macrohabitat .....

Microhabitat .....

F. Method of storage until inoculated .....

IV. Method of isolation from original source: Date inoculated ..... Diluent used .....

A. Animal ..... Embryonated egg .....

Age ..... Vol. of inoculum ..... Route inoculation .....

Cell culture: Type of cell .....

Culture vessel .....

Medium composition .....

Vol. medium ..... ml. Vol. inoculum ..... ml. .....

Manner of recognition: CPE ..... Plaques .....

Other .....

B. Validity of isolation: Reisolation: Yes ..... No ..... Not Tried ..... Date .....

Isolation by other method: .....

Homologous antibody formation by original source animal:

....

(Yes) (test(s) used (no) (test(s) used (not tested)

Other reasons for validity of isolation .....

.....

V. Other isolations in same area: total to date by year and month .....

.....

.....

Sources .....

Method(s) .....

VI. Properties of virus:

A. Physical: Filterable ..... Type(s) filter .....

Size ..... How estimated .....

Other Information: .....

.....

B. Resistance to chemicals:

Chemical	Method (Dil. virus before or after exposure to chem.)	Titer of virus before exp.	Titer of virus after exp.
Ether	.....	.....	.....
Sod. desoxycholate	.....	.....	.....

Other .....

(See reverse side for instructions)

This Catalogue is for the purpose of registering animal viruses that are biologically transmitted by arthropods and are actually or potentially infectious to man or domestic animals. Consequently, no virus should be submitted for registration unless there is some presumptive evidence that it meets these qualifications, or is antigenically related to one that does.

The questionnaire should be filled in with a typewriter, using a relatively new black ribbon and clean type and erasures avoided. This request is made because a photostatic plate will be made from the filled-in questionnaire for transfer to the card to be included in the Catalogue. Please keep within margins. You should retain a copy of the filled-in questionnaire for reference in case any change or additions are suggested.

If question cannot be answered for lack of knowledge or because the examination was not made, please so state: "not known" or "not done" as may apply.

If in answering a question or in giving data it is desired to make reference to a published article or to a personal communication, do so by number and list reference under the corresponding number on page 4. To economize space do not give title of published articles and give only first, or possibly second, author's name.

Some of the questions that may require clarification will be taken up in order.

I. Virus name and/or number. If a record of the virus has been published, use or give reference to the name or designation that was employed in the publication. If no publication has been made, please designate the virus by name or number for at least temporary reference. If the virus is given a name and if more than one strain has been isolated, it is desirable to identify the prototype strain.

II. Antigenic: Group. Refers to the groupings A, B, C, etc., as suggested by Casals. If the virus falls into any of these groups, specify the group, but if it, to your knowledge, has not been grouped, record "Yes" under ungrouped. For recording further antigenic information, refer to VI C.

III. Original source. This refers to the original isolation of prototype strain.

B. Genus and species. Give name of the vertebrate from which the virus was isolated. For arthropods, the genus and species name should be given. For vertebrates the common name may be used but should be followed by the scientific designation. If the virus was recovered from a sentinel animal, be sure to so indicate.

Age or stage. Age, of course, applies only to vertebrates and, except for man and sentinel animals, the exact age may not be known. If the exact age is not known, use terms generally applicable, such as infant, nestling, immature, young adult, adult, etc.

Stage: Applies to arthropods, especially to such as ticks; specify if larva, nymph, adult, etc. Give sex if it can be or was determined.

Isolated from: Blood, etc., is obviously applicable to vertebrates only.
Signs and symptoms of illness: If the isolation was made from man or some lower vertebrate, give signs or symptoms of illness if known. If from autopsy material, so state. More complete information on the nature of the disease, particularly in man, may be recorded under VI B.

D. Method of collection. This applies mainly to lower animals and arthropods, though if obtained from man at autopsy, it should be so indicated. For wild animals or birds, indicate whether shot, trapped, netted, etc., and for arthropods whether caught by hand, trapped, kind of bait, etc. If arthropods were collected from animals, give species of animal from which collected.

E. Geographical: Refers to country and political sub-division within the country such as state, county, city, town, etc. In addition the approximate latitude and longitude may be given.

Macrohabitat: Under this heading indicate if collected in rural or urban community, type of terrain, vegetation, etc.

Microhabitat: Refers to the more exact place of collections such as whether indoors, out of doors, elevation or ground level, character of vegetation, in sun or shade, etc.

F. Method of storage until inoculated. This applies only if the specimen was not inoculated until sometime after it was taken. If it was held for some time before it was inoculated, indicate container and storage temperature.

IV. A. Method of isolation from original source: The questions under this heading should be sufficiently clear and require no explanation. Here, as in all other instances, if there is not sufficient space, additional information may be given at the end of the questionnaire under "remarks" or by references to published articles.

IV. B. Validity of isolation. Applies particularly to new viruses whose identity and disease-producing propensities have not been definitely determined. In such cases evidence should be furnished on the validity of isolation. If the specific questions asked cannot be answered, or the tests were not made, give other reasons why it is believed the isolation was valid, such as repeated isolation from similar or other sources and presence of specific antibody in associated vertebrates.

Homologous antibody formation by original source animal: Will apply to man, sentinel animal or instances where the animal was retained alive or recaptured and it was possible to secure a second or "convalescent" blood sample for testing against the virus isolated.

V. Other isolations in same area. Again this applies particularly to newly-reported viruses.

VI. Properties of virus. These questions should be sufficiently clear, but if the tests have not been made, be sure to indicate "not done". Further characteristics of the virus which are deemed important, such as susceptibility to temperature changes, desiccation, etc., should be recorded under "Other information".

Under Resistance to Chemicals a space is left for recording reaction to any other chemical such as trypsin that may have been tried.

C. Antigenic: Hemagglutinin produced? Yes ..... No ..... Not tried .....

Source material used .....

Methods employed .....

Source of erythrocytes .....

pH: Range ..... Optimal ..... Temp: Range ..... Optimal .....

Antigenic relationship and lack of relationship to other viruses:

Methods found most useful for investigating antigenic characteristics:

Comments and additional information:

(See reverse side for instructions)

In furnishing information on hemagglutinin give preferable source material and method of preparation of antigen. If no hemagglutinin has been demonstrated give methods that were tried.

In describing relationship and lack of relationship with other viruses, be as specific as space and data permit. Be sure to specify method (HI, hemagglutination inhibition; CF, complement fixation; NT, neutralization; PC, protection challenge, that is immunization and subsequent challenge with live virus; other methods). Also, information should be given on the animal source and method of preparation of the immune sera employed, i.e., schedule of inoculation and bleeding.

If the agent has been assigned to an antigenic group, give in detail results of comparison with other members of the group. These data should, by preference, be tabulated giving the homologous and heterologous titers according to the method employed.

If the agent has not been assigned to an antigenic group, list the viruses with which it has been compared, the methods employed and the results obtained.

If data are too extensive to record in detail, as may be the case with well known published viruses, summarize and give references to publications or other reports that may be available.

The antigenic information given should apply to the prototype strain. If considerable antigenic variations have been observed in different strains of the same virus in nature or as a result of laboratory manipulation, this may be noted.

While the recorder is at liberty to choose the method he desires of presenting data, for sake of uniformity the following method of tabulation is suggested:

IMMUNE SERA	Antigen of Registered Virus						ANTIGENS	Immune Serum of Registered Virus					
	HI		CF		NT			HI		CF		NT	
	$\frac{Ht}{Ho}$	Indx	$\frac{Ht}{Ho}$	Indx	$\frac{Ht}{Ho}$	Indx		$\frac{Ht}{Ho}$	Indx	$\frac{Ht}{Ho}$	Indx	$\frac{Ht}{Ho}$	Indx

In the table, no provision has been made for recording the titer of the antigen. It is presumed, however, that the same number of HA units of antigen will be used in the HI test for titrating the homologous as the heterologous immune sera. If the titer of the CF antigen is of special interest a note to this effect may be added, otherwise the titer of the immune serum will be recorded as the maximum titer irrespective of the amount of antigen employed.

Ho = homologous titer, or titer of an immune serum with its own antigen or virus.

Ht = heterologous titer, or titer of an immune serum with another antigen or virus as indicated.

For HI and CF, the titer is the reciprocal of the dilution endpoint of the immune serum.

Indx = Index, obtained by reduction of the fraction by dividing the Ht titer into the Ho titer. Use of Index is optional.

For NT the titer represents the amount of virus neutralized as expressed in logs.

The left half of the table is for recording the results of titrations of immune sera of the viruses listed in the vertical column to the left with the virus being registered. The right side of the table is for recording titrations of the immune serum of the registered virus with the viruses listed in the corresponding vertical column. For example, if the HI homologous titer of an immune serum of a virus listed in the extreme left hand column is 1280, and the heterologous titer of this serum with antigen of the virus being registered is 640, then enter in column under HI, $^{640}/_{1280}$ and in the Ind column ½, indicating that the heterologous titer is ½ that of the homologous. If, in the right hand portion of the table, which represents titers with the immune serum of the virus being registered with the antigens listed in the column to the left, the homologous titer is 640 and the titer with the antigen of the virus against which it was tested is 80, then there should be entered under HI, $^{80}/_{640}$ and in the Ind. column ⅛. The same would apply for recording the results of the CF tests.

For the NT, the logs neutralized both in the homologous and heterologous titrations should be entered but due to the inherent inaccuracies of the NT and the questionable significance of minor differences in titrations, it seems inadvisable to attempt to establish an index of comparison.

PC refers to immunization with the registered virus and challenge with the same and other viruses with which it has shown a relationship by serological tests. The results of PC tests may be recorded separately or in the NT column.

The table is mainly for recording results of comparison with viruses in the same antigenic group or with viruses with which some relationship has been demonstrated. For ungrouped viruses, and where comparison with other viruses has given entirely negative results, it will suffice to enumerate the viruses with which comparison has been made, giving the method or methods that were employed, and the homologoous titers of the immune sera.

D. Biologic properties:

Natural host range

Animal and arthropod	No. virus isolations/no. hosts tested	No. with antibody/no. tested (include type of antibody)

Experimental host range (animals; arthropods; embryonated eggs: (TC) cell culture)

Experimental host	Passage history strain used	Age of animal or egg	Inoculation Route	ml	Evidence of infection	AST	Titer (logs) (vol)
Mice		1-4 day	ic				
"		1-4 "	ip				
"		3-4 week	ic				
"		3-4 "	ip				

Remarks:

(See reverse side for instructions)

VI. D. Biologic properties. Here should be listed all vertebrates and arthropods from which the virus has been recovered in nature, as well as results of antibody surveys, giving number tested and number positive. Also, under remarks, the type of disease, symptoms, etc., produced in animals, and especially in man, should be given. It may also be well to indicate what is believed to be the most likely cycle in nature. For the better known viruses on which there is much published information, reference to published articles may be made, if there is not sufficient space for recording incriminated vectors and vertebrate hosts.

Experimental host range. Give animals, arthropods, embryonated eggs, etc., as well as cell cultures (type of cell) that have been tested for susceptibility to infection with the virus. Give passage history of strain used in test: e.i. number of passages in animal (species) or TC. Give evidence of infection (such as in animals, paralysis, death, etc.) and (AST) average survival time. Since it is frequently useful in classifying the viruses, behavior in infant and adult mice should be recorded. If record of cell culture is given, indicate whether it produces CPE (cytopathogenic effect and/or plaques). If the information cannot be recorded in the limited space, give references to publications or other reports. Titer refers to the infectious titer in the animal or TC desginated, preferably expressed in infecious units per ml.

Histopathology:

Known geographic distribution: by virus recovery:

Suspected geographic distribution: from serological surveys:

Subsequent information:

References:

Remarks:

(See reverse side for instructions)

Histopathology: Give briefly information on tropism and lesions produced in both experimentally and naturally infected animals, with notation of inclusion bodies or any other highly characteristic pathological reactions.

Geographic Distribution: Designate under "Virus recovery" or under "Serological surveys", as may apply, the continents and countries involved. For more detailed information, for which there is not sufficient space for recording, give references to published data.

Subsequent information: It is hoped and expected that the reporter of this virus will supply the editor of the virus catalogue with additional information, either direct or through references, as it may become available. This applies to all properties and behavior of the virus such as additional evidence on its antigenic relationships, host range, general ecology, epidemic outbreaks, geographic distribution, etc.

References: List reference as referred to by number in preceding items. Save space by not giving title of published articles and only the name of first or possibly second author.

Remarks: Space is left here for any additional information of importance not covered in previous questions or where space was not sufficient for recording. But, do not use this space unless necessary as it may also be employed for approved additions to the preceding records.