ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number One April 1960

Table of Contents

INTRODUCTORY NOTES FROM THE SUB-COMMITTEE ON INFORMATION EXCHANGE

This first newsletter on American Arthropod-borne Virus Research inaugurates one service charged to the Sub-committee on Information Exchange, organized under authority of Section Five of the 1959 Gould House Conference on Arthropod-borne Virus Investigations. It is not as comprehensive nor does it contain as great a variety as would be possible or useful under the intent of those who originally suggested or favored such a publication. On the other hand, it contains primarily information summaries of annual and other official reports, contributed specifically for this newsletter by a large percentage of investigators invited to do so. It brings together in one place key information about a wide variety of projects and accomplishments of American-sponsored, financed, or associated arthropod-borne virus research, covered by extensive reports which have been received. It indicates existence and content of these reports, which are listed subsequently in this newsletter, should readers wish to consult certain of such sources in detail.

Contributions by foreign investigators and review of arbor virus work of rapidly increasing foreign laboratory and field activities is absent, being clearly beyond the responsibility and authority of the American group as organized so far. There is also lacking much in the way of reports on current or new field investigations or laboratory techniques. These and other shortcomings, in contrast to the material of interest and value which does appear, may stand out sufficiently to stimulate contributions of the indicated sort for the next issue.

It appears now that the scope, interest, and value of the comprehensive coverage of annual report and project summaries received during the first quarter following the year of reported accomplishment is sufficient to provide the main substance of one issue of the newsletter each year; the other one or two published in fall and/or winter would deal more with current news on field studies, interim developments, and occurrences of immediate epidemiological interest.

As concluded in the initial letter of solicitation for contributions sent last December, the content, size, style, and utility of the newsletter is up to those who contribute to and receive it. This charter group of investigators and agencies as now constituted is listed at the end of this issue.

Concurrent with the debut of the newsletter, the first part of the punch card Catalogue of Arthropod-borne Viruses is issued. Containing records of more than 49 strains, many of them new and unpublished, the catalogue enters the field as a preliminary effort to bring together in one place the latest salient information on a rapidly growing number of arthropod-borne viruses in a form that can keep a variety of globally scattered investigators up to date, with a minimum of duplication of work and without prejudicing later formal publication of the comprehensive detail required of new virus strain reports.

Here again this pilot endeavor has necessarily been confined primarily to American effort in its initial stages. But it is obvious that to

be of greatest value and utility it must be extended to international use and participation.

If the material results in the way of newsletter and catalogue are worthy of the objectives set forth by the Gould House Conference and efforts of the Sub-committee, then the participants, contributors, and recipients must next give consideration to improvement and expansion of these instruments through extension to and more active participation by a globally representative group.

The catalogue is obviously incomplete at this stage, nor is it ever expected to be really complete at any point in time, since it is an instrument designed to keep abreast of isolation and characterization of new arbor viruses and to accumulate more knowledge about those already recognized. Now that the design, punch card system, coding, and reproduction methods have been worked out, it is expected that each month will bring issue of new cards representing not only newly reported viruses but also additional information for standard strains until this section of the catalogue is complete.

Finally, all that the Sub-committee on Information Exchange has implemented so far as been in response to the tasks assigned to it by the Gould House Conference. While workable instruments such as the newsletter and punch card catalogue have been devised, they do not necessarily represent any final form. Corrections and suggestions for improvement are hereby solicited.

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REPORT FROM DR. RUSSELL ALEXANDER, EPIDEMIOLOGY BRANCH,
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For the past five years, during the season and in an annual summary, the Communicable Disease Center of the U.S. Public Health Service has distributed Encephalitis Surveillance Reports. This information source has proved useful to many persons engaged in the investigation and control of arthropod-borne encephalitis. The report consists of a brief preliminary analysis and summary of the occurrence of the arthropod-borne encephalitides in the United States. All readers of this Newsletter are encouraged to write to the Surveillance Section, Communicable Disease Center, 50 Seventh Street, N.E., Atlanta 23, Georgia, if they wish to be placed on the mailing list for these reports.

The Summary Report for 1959 has not been completed, due to delay in receipt of information on laboratory proven cases, but the report should be ready by June, 1960. Following is a summary of events of 1959.

In 1959, preliminary reports indicate an unusual occurrence of the arthropod-borne encephalitides. Of greatest interest was the occurrence of Eastern Encephalitis (EE) on the Atlantic Seaboard. In humans the disease occurred in epidemic form in New Jersey from August 17 to October 15, affecting 33 persons. As usual, the fatality was high, and to date 21 cases have died. Extensive studies of the factors responsible for this occurrence were begun in 1959, and long-term studies will be continued. Isolated cases were also reported from Maryland and Florida.

It is of interest to note the numerous reports of virus isolation from Massachusetts to Trinidad in 1959. In Massachusetts, virus isolation was reported from pheasants (Hayes). In Connecticut EE was isolated from Aedes vexans, as well as from pheasants and horses (Wallis, Smith and Taylor). In New York unusual occurrence was noted by recovery of this virus from ducklings in Long Island (Overton). In New Jersey EE was isolated from mosquitoes (Culex restuans and a mixed pool of Aedes species), wild birds (English sparrow and myrtle warbler), captive pheasants, horses and man (Kandle, Hayes, Stamm). In addition, virus was isolated from wild birds (English sparrows) in Maryland (Herman) and from pheasants in Pennsylvania (Witte). In Alabama, continued studies revealed evidence of the virus in Culiseta melanura and Aedes vexans in November, and in a wild bird (white-throated sparrow) in December (Chamberlain, Stamm). In Trinidad, EE was isolated from Culex nigripalpus in May and Culex taeniopus in August and September (Downs, Aitken and Spence).

St. Louis encephalitis occurred in small concentrations, with the largest group (32 cases) occurring in California during the latter part of the season. Of unusual interest was the recurrence of this disease in Florida. Although isolated cases have previously been discussed, an outbreak was recognized for the first time in St. Petersburg in October, 1959. The final total of cases will rely on late laboratory results for serologic confirmation, but at least 30 cases occurred, in an epidemiologic pattern similar to that seen in urban outbreaks of SLE in central United States. No vector was established by virus isolation, but C. nigripalpus was suspected.

Western encephalitis was notable for its relative absence. There were no reports of outbreaks and individual cases were scattered.

We would like to take this opportunity to invite readers of this Newsletter to be added to our mailing list, and to participate actively in exchanging information relative to encephalitis occurrence in the United States, or neighboring countries. In no sense are we asking for premature release of scientific observations or research studies that will be published separately. Rather, the Surveillance Report will represent a consolidation of the basic facts as to the type and distribution of the encephalitides. In consolidating the data, we are anxious to give full credit to the proper sources.

REPORT FROM DR. ROY CHAMBERLAIN, VIRUS AND RICKETTSIA SECTION, LABORATORY BRANCH, COMMUNICABLE DISEASE CENTRE, MONTGOMERY, ALABAMA

Current Study, South Alabama Field Area, near Bay Minette, Ala. (Baldwin County)

A field study in south Alabama was initiated in November, 1959, to determine whether EE or WE viruses overwinter in that area through continuous transmission to birds by mosquitoes. A study of this same area in 1957-1958 had revealed strong virus activity in birds and mosquitoes, and EE neutralizing antibodies in approximately 10 per cent of 140 humans tested.

In the current study, birds are being netted, banded, bled and released according to a standardized pattern and schedule, using 21 Japanese mist nets placed 200 feet apart on two intersecting 2,000-foot lines. Pens of sentinel chickens and wild birds were also established, to be bled each month for serological tests.

Between November 4-17, 5 isolations of EE virus were made from mosquitoes, 4 of which came from Culiseta melanura and one from Aedes vexans; at one sampling period (November 4 and 5) approximately 10 per cent of the C. melanura were infected (3 isolations were made from 33 mosquitoes tested). Two isolations of WE were also made in November, one from C. melanura and one from C. salinarius.

A total of 156 birds were captured, banded and bled in November. An isolation of EE virus was made from a tufted titmouse on November 17 and from a rufous-sided towhee on November 18. An additional 257 birds were collected and tested in December (up to January 2, 1960), with EE virus obtained from a white-throated sparrow on December 27, and again from another white-throated sparrow on December 28.

No viruses were isolated from 668 birds tested in the remainder of January and February, 1960. Serological tests on these birds for evidence of antibody conversion have not yet been completed.

No viruses were isolated from mosquitoes collected in December. In January and February the mosquito collections were essentially nil because of cold weather.

The study will be continued into next fall with the objectives of relating the rate of virus transmission to local bird density as it varies seasonally due to migration and reproduction, and to mosquito population. A comparison of antibody conversion in netted wild birds and sentinels will be made in attempts to correlate results in captive and free populations.

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

Most of the encephalitis research work of U.S. Public Health Service's Communicable Disease Center is now consolidated in the new Encephalitis Section headquartered at Greeley, Colorado. The Section is primarily concerned with field and laboratory investigations on the natural history and control of eastern, western, and St. Louis encephalitis. Dr. A. D. Hess is Chief of the Section; Dr. Louis C. LaMotte is Chief of the Virology Unit; and Dr. James V. Smith is Chief of the Biology and Control Unit. In addition to Greeley, the Section maintains three small field stations: one at Bakersfield, California, under Dr. R. Edward Bellamy; one at Wenatchee, Washington, under Virgil I. Miles; and one at Taunton, Massachusetts, under Dr. Richard O. Hayes.

The principal facilities at Greeley include a main office and laboratory building, a biology building with mosquito insectary and invertebrate laboratory, a vertebrate laboratory (just completed), and animal rearing house, a shop, and several warehouses.

Encephalitis Sentinel Shed and Mosquito Trap

The CDC Encephalitis Section at Greeley, Colorado, has developed a demountable chicken sentinel shed and mosquito baffle traps for use in encephalitis field studies. These units provide information on encephalitis transmission rates, mosquito populations, and mosquito infection rates.

The shed has inside dimensions of 6 x 7 feet, with a 7 x 14 feet fenced enclosure in front. For transportation or shipment each shed is made up into two bundles, one 4' x 8' x 11" and one 4' x 7' x 9". Two removable mosquito traps with modified Egyptian-type baffles fit into a horizontal opening beneath the front eaves of the shed. Mosquitoes collected from these traps provide a population index and an estimate of the mosquito attack rate, and they may also be used for virus isolation tests. Thirty to fifty chickens are kept in each shed during the summer. They are bled in the fall and their sera are tested for encephalitis antibodies; the per cent positive provides an index of encephalitis transmission. During 1959 sentinel sheds of this type were maintained at Bakersfield, California; Columbia Basin, Washington; northern Utah; Grand Junction and Greeley, Colorado; Fargo, North Dakota; the panhandle and lower Rio Grande areas of Texas; and Taunton, Massachusetts. Over a period of years it is believed that these sentinels will provide much valuable information on the transmission patterns of SLE, WE, and EE and the various factors which influence these transmission patterns.

The sentinel shed and its uses were described in a paper to be presented by members of the Greeley Field Station staff at the annual meetings of the American Mosquito Control Association in Boston, Massachusetts, March 28, 1960.

REPORT FROM DR. W. C. REEVES, SCHOOL OF PUBLIC HEALTH,
UNIVERSITY OF CALIFORNIA,
BERKELEY

Summary Statement

Studies of the overwintering of Western equine and St. Louis encephalitis viruses indicate that both Culex tarsalis and chronic-latently infected avian hosts may be important reservoirs of infection.

Differentiation of C. tarsalis virus infection and transmission rates indicate both are important in epidemiological field studies. Field data on viremia incidence and antibody prevalence in birds are compatible with data on vector infection and transmission rates.

WEE and SLE viruses were maintained for one year by 7 serial C. tarsalis to chicken to C. tarsalis transmissions at the ambient temperature of an endemic area.

Wild bird species of the endemic area vary in viremia titers following virus incubation.

In 1958, an epidemic of WEE threatened in Kern County. Epidemiological studies indicated a combination of low June temperatures followed by intensive vector control minimized the number of human cases.

Three study areas are selected for demonstration of the effect of vector control on virus transmission. Preliminary data on vector population virus activity and bird populations indicate these will be acceptable areas.

Supporting studies are in progress on statistical methods for calculating vector infection rates, identification of bird blood, autogeny and physiological age of C. tarsalis, and evaluation of HAI and tissue culture procedures for epidemiological studies.

Intensive vector control was begun in one area in February, 1960, and will be continued for at least a 2-year period.

Studies of WEE virus plaque formation indicate a potential difference between the characteristics of freshly isolated field strains and laboratory adapted strains.

A cooperative short-term field program on arthropod-borne viruses is being carried out at Hermosillo, Mexico, in March and April. Cooperating agencies are the Mexican Government, California State Department of Public Health,

Rockefeller Foundation, and School of Public Health, University of California. The project has the combined objectives of training and a general survey for virus activity. The program will terminate in a field demonstration for members of the Mexican-American Border Public Health Association.

REPORT FROM DR. E. H. LENNETTE, CHIEF, VIRAL AND RICKETTSIAL DISEASE LABORATORY, CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH, BERKELEY

STUDIES ON ARTHROPOD-BORNE VIRUSES 1959 - 1960

Investigation on Animal Reservoirs of Human Disease

A. Animals and Ectoparasites

This study is directed toward the discovery of small mammalian reservoirs of viruses pathogenic for man. The investigation is a cooperative undertaking with the Bureau of Vector Control, California State Department of Public Health, involving a mammalogist and an entomologist.

An approach to the problem has been to trap (alive) small mammals in two separate areas, viz., the Napa Valley in the central coastal area, and Sagehen Creek in Sierra County, 12 miles north of the town of Truckee. These locations were chosen because Western equine encephalomyelitis virus (WEE) has been isolated from squirrels in the Napa Valley region and Colorado tick fever virus is known to be present on the east side of the Sierra Nevada Mountains.

Trapped animals are anesthetized and a blood sample collected. Spleen, kidney, and a piece of large intestine are removed and frozen for future examination. Suspensions of spleen and kidney pools are inoculated into embryonated hens' eggs and 1-2 day old suckling mice for virus isolation. The sample of intestine is collected for a potential study of the enteric viruses of small mammals.

At the end of calendar 1959 a total of 230 animals had been trapped. The species and number that were collected are as follows:

Brush mouse (*Peromyscus boylei*)	11
Deer mouse (*Peromyscus maniculatus*)	59
Pinyon mouse (*Peromyscus truei*)	54
White-footed mouse (*Peromyscus californicus*)	3
Pocket mouse (*Perognathus californicus*)	1
Wood rat (*Neotoma fuscipes*)	12
Kangaroo rat (*Dipodymys hermani* and *vanustus*)	11
Ground squirrel (*Citellus beecheyi*)	10
Golden mantle squirrel (*Citellus lateralis*)	9
Chipmunk (*Eutamia amoenus*)	26
Pallid bat (*Antrozous pallidus*)	23
Freetail bat (*Tadarida brasiliensis*)	11
	230

All of the tissue and blood samples from animals collected in the Sierras have been tested for evidence of infection with Colorado tick fever virus. The serum of one chipmunk (Eutamia amoenus), trapped near the Truckee River, 4 miles east of the town of Truckee, neutralized 1.5 logs of Colorado tick fever virus. All of the other specimens have proved negative.

No agent has been isolated from the tissue specimens from animals collected in the Napa Valley area. Sera from these animals examined for antibodies to Western equine encephalomyelitis and St. Louis encephalitis viruses in a tissue culture neutralization test have all proved negative.

During the period covered by this report, a total of 26 pools of ectoparasites has been examined for the presence of an infective agent. Pools consisting of from one to 20 adult ticks were ground into a suspension in a mortar, and inoculated into embryonated hens' eggs and 1-2 day old suckling mice. All of these pools consisted of ticks collected either from trapped animals or by flagging. The species tested were:

Dermacentor andersoni	12 pools
Dermacentor occidentalis	8 pools
Dermacentor perumapterus	4 pools
Haemaphysalis leporis	1 pool
Ixodes sculpus	1 pool

Colorado tick fever virus was isolated from two pools of Dermacentor andersoni ticks. One pool originated from Portola in Plumas County and the other was collected at the Hansen ranch in Modoc County. All of the other ectoparasite pools yielded negative results.

B. Arthropod-borne Viruses

During the summer of 1959 an attempt was made to learn whether the Culex tarsalis mosquito is the most important vector of the St. Louis encephalitis virus. Mosquitoes for testing were collected in two areas (Laton and Riverdale) in Fresno County. Only previously fed females were tested in those instances where mosquitoes were plentiful.

Of 82 pools made up of species other than C. tarsalis (C. pipiens, C. peus, An. franciscanus, An. freeborni and Cu. inornata) that were tested, 81 failed to yield the St. Louis virus or any other viral agent in the hosts employed; the one remaining pool (C. peus) yielded an agent which thus far has defied identification. Further study will be required to identify this agent.

As control material, isolation procedures were also carried out on 41 pools of C. tarsalis collected concurrently with the pools of other species. Of these, 37 were negative, three yielded a St. Louis virus, and one a Turlock virus. It would appear from this small sample that infection with St. Louis virus is more prevalent in C. tarsalis than in the other species of mosquitoes.

In diagnostic procedures performed by the laboratory during the 1959 season, serologic evidence of infection with Western equine encephalomyelitis virus was shown in one individual, and with St. Louis virus in forty.

St. Louis encephalitis virus was recovered from the brain tissue of a 65-year-old male who had worked in the rice fields of the Sutter-Yuba area for the past 16 years. It still remains an enigma that the St. Louis encephalitis virus should frequently cause a fulminating encephalopathy in older individuals, who because of their many years of exposure to the vector Culex tarsalis mosquito, might be presumed to have built up an immunity to this agent.

REPORT FROM DR. R. M. TAYLOR, SECTION OF EPIDEMIOLOGY AND PREVENTIVE MEDICINE, YALE UNIVERSITY MEDICAL SCHOOL, NEW HAVEN, CONNECTICUT

1) Ecologic and Epidemiologic Studies on EEE

For the first time since 1956 Eastern equine encephalomyelitis was manifest in horses and pheasant farms in Connecticut. According to data furnished by the Veterinary Diagnostic Laboratory of the University of Connecticut, it was confined mainly to the south-central portion of the state, and to foci in the proximity of swampy, wooded areas. No human infections were recognized.

In view of the inactivity of infection during the past two years and the inability to obtain services of an ornithologist during the summer, the collection of birds was much less extensive this past year than during the previous two years. In consequence, little knowledge was obtained on the infection of wild birds. Bloods were obtained, however, from 82 crows and rather surprisingly EEE neutralizing antibodies were detectable in only ten per cent. It should be emphasized, however, that these crows were collected at a winter roosting site, and it is quite likely that most of them may have spent the summer season further north and, therefore, were not representative of this area.

The principal significant contribution from our studies was the isolation of EEE virus from Aedes vexans. This mosquito on ecological grounds had been suspected as a transmitter in this region. The isolation of the virus gives support to this conception. It is more cosmopolitan in its feeding habits than Culiseta melanura and may be important in transmitting the infection to vertebrates other than birds, such as horses and possibly to man, although human infections were unrecognized.

Notwithstanding the susceptibility of certain non-hemophagous insects to infection with EEE, efforts to demonstrate the passage of the virus from one generation of the insect to the next have been unsuccessful, so far. Nor was it possible to demonstrate virus in non-hemophagous insects captured in the vicinity of infected pheasant pens.

2) Studies in Tissue Culture

A comparison of the sensitivity of selected viruses to diethyl ether and sodium desoxycholate demonstrated parallel behavior to these two agents and confirmed the opinion that under certain conditions either of these chemical

methods may be useful in categorizing virus isolates. Both inactivated ten arthropod-borne viruses and two myxoviruses. In contrast, poliovirus, two Coxsackie, three ECHO viruses and three adenoviruses were not inactivated by either diethyl ether or sodium desoxycholate.

Primary cell culture infectivity spectra have been established for 28 arthropod-borne viruses comprising sero-groups A, B, C and certain ungrouped viruses. Chick embryo, Pekin duck embryo and rhesus monkey kidney cell culture systems have been utilized. Duck embryo monolayer cultures have been useful in propagating 24 arbor viruses included in A, B, C, Bwamba, Quaranfil, Bunyamwera and California sero-groups.

Mouse kidney cell cultures are under study for use in the Sand-fly fever virus neutralization test. Preliminary studies with 23 human sera collected in Cairo showed that two of the 23 samples neutralized 1 to 2 logs of H-5202 (Sicilian) virus.

Interference studies utilizing certain arthropod-borne viruses or their nucleic acid counterpart as interfering agents have been undertaken. When Mayaro virus is subjected to treatment with sodium desoxycholate and absorbed to chick embryo monolayer cultures, the multiplication of other superinfecting arbor viruses is altered. The interfering effect of SDC-treated Mayaro could not be demonstrated in the presence of RNAase. Attempts to demonstrate an "interferon" have been unsuccessful.

Contemplated projects for 1960

Ecology of EEE

Observations, collections and laboratory examinations of mosquito and bird specimens will be continued during the year. Selected sites in Connecticut with a history of EEE endemicity will be observed, and periodic collections of mosquitoes and birds will be examined in the laboratory. As in the past, this will be a collaborative project with Dr. R. C. Wallis of the Connecticut Agricultural Experiment Station and members of the State Board of Fisheries and Game.

Projected Survey for Arthropod-borne Viruses in Florida

While there is evidence that St. Louis, Eastern and perhaps Western encephalitis viruses have given rise to human infection in Florida and that Eastern virus infection is common in horses throughout the state, the possibility of other arthropod-borne viruses being present has not been adequately investigated. It is felt that a survey for other viruses is indicated because a considerable proportion of the cases of human encephalitis remain undiagnosed as to their exact etiology and also because of the possibility of the entrance of "new" viruses from South and/or Central America. During the past few years a score or more of hitherto unrecognized arthropod-borne viruses, infectious to man, have been isolated at laboratories in Trinidad and Belém. The Florida peninsula is the nearest geographical link with South America and is on the flyway of migratory birds as well as being the principal port of entry of man traveling by air. Thus it should be the most likely place of introduction of infectious agents transported by either birds or man.

Since most of the viruses that have been found in South America are not pathogenic to adult mice during early passages, field studies on EEE employing only adult mice, such as the very excellent one made by Major F. G. Favorite in cooperative with Dr. J. E. Scatterday, Florida Department of Public Health Veterinary Officer, would not reveal the existence of most of the new South American viruses.

The present survey, which is being conducted with the permission and in cooperation with the Florida State Health Department, is of a preliminary and temporary nature and will terminate at the end of the summer. It is hoped, however, that the results may be useful in planning more long-term investigations. Both serological and virus isolation methods will be used. For the first it is hoped to secure, with the assistance of Dr. T. H. Work, a representative sample of bloods from the Seminole Indians and perhaps some from other permanent residents in the area, and examine for HI antibodies, using a large battery of antigens including all known important South American viruses. These sera will be examined in The Rockefeller Foundation Laboratories in New York under the supervision of Dr. Work. For virus isolation, reliance will be placed largely upon sentinel infant mice which have proved so successful in revealing the "new" arthropod-borne viruses in South America. In addition, arthropods and a limited number of bird bloods will be collected at the sites where the sentinel infant mice are stationed.

Doctors Chamberlain and Stamm of the Virus and Rickettsia Section, Laboratory Branch, Communicable Disease Center, are collaborating with the Florida Department of Health on bird and mosquito investigations at Sawgrass Lake, north of where an Eastern and St. Louis virus encephalitis epidemic occurred in Pinellas County in the summer and fall of 1959. It is expected that these two Florida investigations will be usefully complementary.

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

A Collaborative Field Study of EEE in Panama

In August, 1958, the laboratory assisted Major T. Murnane (U.S. Army Mission to Panama and Laboratorio Veterinario R. de P.) in identification of a viral isolate from a suspected equine case of encephalitis histologically confirmed as viral encephalitis at Gorgas Memorial Laboratory (GML). After the second isolation of what proved to be the EEE virus GML established a field station near the affected corral in Pacora (40 miles from Panama City) which was operated for two months with the assistance of MARU. During the initial field study the GML field staff selectively collected and identified more than 25,000 mosquitoes belonging to at least 11 different genera. The frozen pools were tested for virus isolation in suckling mice and tissue culture in the two laboratories. With the advent of the dry season the collections were suspended, but as the mosquito population began to increase in April, 1959, the study was resumed with the assistance of the U.S. Army Malaria Control Team while the GML staff was temporarily occupied with other commitments.

In August, 1959, two horses dying of clinical encephalitis were seen by Major Murnane near the Tocumen Airport, R.P., and Curundu, C.Z., respectively. The brains were repeatedly examined for viral isolation with negative results, but histological examination of both brains at GML revealed extensive encephalitis consistent with viral infection. The other common causes of encephalitis in this area were reasonably ruled out. The terminal "acute" blood specimens from both animals did not contain demonstrable antibodies against EEE virus by neutralization or complement-fixation tests, but both had high hemagglutination-inhibition titers (1:20,000 and 1:40,000). The Pacora site was temporarily abandoned; the nearby Tocumen area was covered by the GML staff, while the Malaria Team collectors shifted their activities to the Canal Zone.

The virus of EEE has not been isolated from any of the arthropod pools, in spite of inoculation into suckling mice (at MARU and GML), and the tissue culture systems of hamster kidney and chick embryo. From several mosquito pools, particularly Culex dunni, other ARBOR viruses were isolated at GML and MARU; their nature as yet has not been fully clarified.

In October and November, 1958, 210 wild birds representing 55 species of 29 families were taken by shooting or trapping by MARU field investigators working jointly with the GML staff (unfortunately, this represented only a small sample of the avifauna in the area as indicated by the bird check lists with over 80 families and 1,400 species known to occur in Panama). All of the avian serum specimens were checked for the presence of EEE virus in chick embryo cell cultures. Practically all of the sera were tested for the presence of HI antibodies after acetone extraction by the method of Casals and Clarke. Sera from six birds inhibited hemagglutination at a dilution of 1:80 or greater and were considered "positive": 2 boat-bill herons, 1 blue heron, 1 meadow lark, 1 flycatcher, and 1 toucan.

Of the 97 horse sera obtained in the Pacora area (Lab Vet), prior to the mass vaccination by the Ministry, 41 were found to contain neutralizing antibodies against EEE virus. Eighty-four of these horses were pastured near the tidal lowlands and 38 of them (45%) contained neutralizing antibodies, while only 1 of 13 horses kept in the stables had antibodies (8%). The difference between the two groups is suggestive of the pasture exposure and points to the mosquito vector with short flight range. Over 100 horses from other areas of Panama have been compared with the base line studies at Pacora with varying incidence of positivity possibly related to similar ecological factors.

Serological studies were performed on 222 sera from residents of 13 villages in and near the Pacora site. Only 3 sera were found positive by neutralization and hemagglutination-inhibition tests. If the population is divided into groups of residents in the savanna terrain (102), transitional terrain (74), and tidal lowlands (46), the percentages of serum positivity are 0.0%, 1.5%, and 3.5%, respectively.

Over 500 CF tests have been run on 187 horse and 209 human sera, primarily from the Pacora study area. Forty-seven of the horse sera were reactive with the EE antigen. The majority of the positive reactors (17/47) was from the horses bled at the time of the outbreak in September, 1958. While a single serum reacted with WE antigen alone, 13 additional horse sera were almost equally

reactive with the EE and WE antigens, suggesting that another group A virus is active in the area. The pattern was different in case of the human sera: 192/209 were negative with both antigens, a single serum was reactive with EE, and 2 sera with WE antigens; 13 sera were reactive with both EE and WE antigens providing further evidence of possibly another group A virus.

The joint studies of the 3 laboratories showed that the EEE virus was not spreading among the horse or human populations to endanger the suburban communities adjoining Panama City and the Canal Zone. It is likely that through the years this virus has been active on the Isthmus of Panama, possibly together with other group A agents. It is suspected that the vector has been a Culex mosquito. The shore birds appeared to be infected more frequently than the inland birds; the actual reservoir of the EE virus has not been established.

Progress Summary of Arthropod Isolate Identification in Panama

As indicated in the summary submitted for the Subcommittee's use in the Newsletter, during the 1958 study of EEE virus in Panama the mosquito pool work was divided between the two participating virological laboratories: the suckling mouse inoculations were carried out by Dr. Enid Rodaniche at the Gorgas Memorial Laboratory and the cell cultures of hamster kidney and chick embryo cells were inoculated at MARU. On one occasion a parallel inoculation of TC and SM was done at MARU for comparative purposes and fortuitously a viral agent was isolated from a pool of C. dunni (B-535). On the other hand, Dr. Rodaniche isolated seven SM agents similar in biological properties all from C. dunni of which one (J-55) was forwarded to MARU for comparative studies. Both viruses were pathogenic for baby mice under six days of age by i.c. route only. The two viruses were soon shown to be serologically distinct.

The B-535 virus was shown at MARU to be DCA (+), noncytopathogenic for HKTC, HeLa, MKTC and human amnion cells; it could not be propagated in 5-8 day embryonated eggs; it was not neutralized by a group B polyvalent antiserum prepared against several common group B agents, attempts to produce hemagglutinating and CF antigens were not successful; attempts to demonstrate antibodies in human and equine sera collected near the area of virus isolation were consistently negative.

During the EEE studies near the Tocumen Airport in 1959 two mosquito viruses were isolated. One was from A. taeniorhynchus and the other from a combined pool of C. dunni and C. virgiltus. Both were pathogenic for suckling mice only and DCA (+); some of the properties were not unlike the B-535 agents described above and it is felt that all of them may belong to one of the newer groups (C, D, Bunyamwera, etc.) by Casals classification.

Since the commencement of a comprehensive project on the ecology of arthropod-borne viruses in Panama initiated by the Gorgas Memorial Laboratory in the Bocas del Toro Province on the Atlantic coast of the Isthmus, all mosquito pools upon identification at GML are equally divided between the two laboratories where they are tested in suckling mice and in addition in HKTC at MARU. So far four arthropod isolates were made at MARU from Bocas specimens. Two of these (BT-78 and BT-104) were made from phlebotomus pools, while the two more recent isolates were from mosquitoes (one from a Psorophora sp. and one

from C. nigripalpus). The two phlebotomus isolates apparently are not identical. The BT-78 agent is of particular interest because it was simultaneously isolated (and subsequently reisolated) in both suckling mouse and HKTC systems. It is DCA (+). A similar agent was isolated in suckling mice by Dr. Rodaniche and upon inoculation (in her laboratory) into HKTC tubes provided by MARU, a characteristic cytopathic effect was observed. The other phlebotomus isolate (BT-104) so far has been noncytopathogenic in any of the TC systems tried. The Psorophora isolate (BT-219) with passage may have been possibly adapted to HKTC.

The work on these isolates (particularly those procured during the last few weeks of 1959) is now approaching the stage where, with procurement of specially prepared known potent antisera from Dr. Pond's "sister" laboratory in Bethesda and production of our own standardized virus pools and homologous hyperimmune sera, we will be in a position to relate these agents to each other and to the known arthropod-borne virus groups. It is hoped to have these results in time for the next Newsletter of the Subcommittee.

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

There were only 23 isolations of ARBOR viruses made during 1959 at the Trinidad Regional Virus Laboratory. A very dry year, particularly in the first nine months, probably influenced this total. However, though small in number, the list is dignified by the isolations of eastern equine encephalitis and Venezuelan equine encephalitis, new members of the Trinidad list of viruses, plus an additional three agents as yet unidentified and possibly new. Also the finding of both eastern equine and western equine in horses in the Rupununi District of British Guiana is noteworthy.

Yellow fever again appeared in Trinidad. After the 1954-1955 episode, no evidence of disease activity had been noted. However, in early 1959, two human cases were picked up in health department outpatient clinics. Both of these cases were in woodcutters from the eastern heavily forested region of the island. Both cases presented as undiagnosed fevers of only moderate severity, and both recovered. Virus was recovered from each. Despite vigilance, no further evidence of yellow fever activity has been uncovered except for the finding of a few monkey carcasses. Despite our failure to observe yellow fever activity in the interval 1955-1959, it is felt that the virus has been on the island continuously, and indeed it is felt it is still present. Failure to uncover it only illustrates our inefficient methodology as far as this disease is concerned, and serves to emphasize that the practice in international quarantine circles of regarding a region as "safe" when no reports come in from it is very short-sighted policy indeed.

Venezuelan equine encephalitis affords another challenging mystery. Present in 1943-1944 in a severe outbreak in equines, it has been absent since. Recent serological studies performed by Colonel Tigertt at the Walter Reed Army Medical Research Center on selected Trinidad sera suggested that this virus or a similar virus, was still about. Since virus studies had been conducted for

six years in the regions he showed to be suspect, it seemed hard to believe that Venezuelan equine encephalitis virus was present, but eluding us despite large numbers of mosquitoes, birds, animals and human clinical cases investigated. However, in late 1959, the virus has been repeatedly isolated from sentinel baby mice and from mosquitoes, in the central part of the Nariva Swamp. It appears here that shifting our collecting site only a few miles, brought this virus to light. Why it may be thus quiet for years, and occasionally move out and provoke a serious island-wide epidemic is still a mystery.

The number of ARBOR viruses isolated in Trinidad now totals 22 distinct agents as follows:*

1) Dengue
2) Yellow Fever
3) Ilhéus
4) St. Louis
5) EEE
6) VEE
7) Mayaro
8) Kairi (TRVL 8900)
9) Manzanilla (TRVL 3587)
10) Oropouche (TRVL 9760)
11) Wyeomyia (TRVL 8349)
12) Cache Valley (TRVL 20659)
13) TRVL 7994 (mosq.)
14) TRVL 8362 (mosq.)
15) TRVL 8762 (mosq.)
16) TRVL 9223 (mosq.)
17) TRVL 9375 (mosq.)
18) TRVL 10076 (mosq.)
19) TRVL 11573 (bat and mosq.)
20) TRVL 18462 (mosq.)
21) TRVL 26668 (mosq.)
22) TRVL 27573 (mosq.)

*Note: Not included for the present is WEE (validity of isolation queried) and TRVL 5843 from a Saimiri monkey (possibly a large virus which has failed to pass a Seitz filter; probably DCA-sensitive).

Virus transmission studies involving mosquitoes have been continued. Successful transmissions using several species of mosquitoes in each case have been obtained with Kairi virus (TRVL 8900), Cache Valley (TRVL 20659) and TRVL 7994. Unsatisfactory results were obtained with EEE, SLE, and Coxsackie B4.

Virus isolations from arthropods, along with EEE and VEE, also include two isolations of the 9223 prototype, our 18th strain of Wyeomyia virus, Coxsackie B4 from Culex, and two isolations of unknown agents. For the first time in six years we have made no recoveries of Ilhéus virus either from mosquitoes or from any other source.

The Philornis fly lead, which looked promising in 1958, has not continued thus attractive after conclusion of a critical experiment in which St. Louis virus viremia in a host nestling and absence of virus in subsequently dropped Philornis larvae has been demonstrated.

A serum survey was conducted in St. Vincent and in the coastal region of British Guiana, and in addition, sizeable collections of serum were received from Curaçao, Venezuela and Surinam. Laboratory analyses were continued on specimens from this year's and earlier surveys, analyses nearing completion for Barbados, St. Lucia, Antigua, Jamaica and the Rupununi region of British Guiana, all surveyed in earlier years.

In the smaller West Indian islands, dengue appears to have been the only insect transmitted virus disease of any prominence in recent years with possibly a limited degree of activity of St. Louis virus on some of the islands. This point is difficult to define clearly because of confusing serological cross-reactions between these two related viruses. Jamaica presents a relatively simple picture with a general high level of immunity to dengue and St. Louis viruses manifest. Trinidad presents a picture of great complexity, on the other hand. Viruses isolated from cases of human disease include yellow fever, dengue, St. Louis, Ilhéus, Oropouche and Mayaro. Additional agents isolated from mosquitoes but not from man include eastern equine, Venezuelan equine, Wyeomyia, Cache Valley, Kairi and other distinct and as yet unnamed ARBOR viruses. Among these latter, immunity in humans has been demonstrated against Venezuelan equine, Cache Valley, Wyeomyia and several other agents, but the disease picture in Trinidad remains unknown.

The surveys of British Guiana indicate a picture comparable to that of Trinidad, as far as the hinterlands are concerned. In addition, the virus of western equine has been recovered from the brain of a horse from the Rupununi Savannah region, and eastern equine was recovered from horse brain from the same region. In the coastal areas of British Guiana, immunity seems to be localized largely to dengue and St. Louis.

In addition to ARBOR virus work, an influenza outbreak in Trinidad was investigated and found to be caused by Asian influenza virus. Kaolin adsorption of sera to be tested appears to be a useful modification of standard influenza diagnostic technic.

Progress was made with tissue culture, using hamster kidney, chick fibroblast and KB cell lines. Hamster kidney cell cultures are being used in routine virus isolation attempts from wild-collected birds. Exploration of the utility of tissue culture neutralization tests done with chick embryo and hamster kidney cultures has been conducted, but such tests have not reached the point of being adopted as routine procedures.

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

Activities and Results, 1959

The investigation of rumored epidemics, the isolation and identification of viruses from vertebrate and invertebrate hosts, and serological surveys continue to be major projects of the program at the Belém Virus Laboratory. The sentinel animal, both monkey and mice, as in past years, has proved to be an efficient source for virus isolation in this area. Improved techniques and enlarged staff at the laboratory have made it possible to process more than 5,500 samples from which were isolated and identified 426 strains of viruses, a larger number of agents than were found in all four previous years of study in this laboratory. Many of these agents were duplicate isolations of already identified strains, but new viruses and double infections are discovered often enough to

make necessary the processing and identification of every isolation. The duplicates involve work equal to the original isolation but may confuse the epidemiological picture if number of isolations and number of infections are not distinguished.

Six new agents were found in the Belém area in 1959, four of these were isolated for the first time during this year, one of them was separated as a new prototype in group C and one was an agent previously described from Colombia.

All of these are interesting viruses but the most intriguing discovery was the sixth member of group C which completes an antigenic circle, and brings order to a previously confusing complex. It had been shown in the RFVL in New York that there were antigenic pairs by neutralization and hemagglutination-inhibition testing that differed from the interrelationships revealed in the complement-fixation test. Now with the uncovering of Itaqui, the previous lone wolf of the group, Oriboca, has a companion that pairs with it in the HI and NT tests, and pairs with Caraparu in complement-fixation testing. The circle is thus closed, as shown graphically in figure 1, and it would seem that any further discoveries in group C should draw the diagram into the third dimension.

The isolation of Guaroa virus from the Belém study areas has dramatic aspects in the three "firsts" associated with its discovery: the first isolation of an arbor virus from the liver by percutaneous biopsy; the first association of a disease with Guaroa in its isolation from four sick forest laborers; and the first discovery of this virus outside of its prototype locality.

The discovery of the fourth member of the Guama group may explain some of the puzzling low titers encountered when typing group Guama strains by neutralization tests. This new virus AR12590 is more closely related to AN277 (Guama) than to H151 (Catu) which is just the reverse of the relationship reported for Tr8362, the group Guama member isolated in Trinidad from mosquitoes.

Both the new group A virus AR13136 and the ungrouped virus AN10615 became more interesting with second isolations, which lend substance to their existence in the area. The single isolation of group A virus Aura (AR10315) is less exciting until there is more evidence of its activity. A start has been made in the investigation by serological surveys of the role each of these viruses may play in the Amazon region.

The development of the serology section of the laboratory has been a noteworthy achievement this year. With the use of the hemagglutination-inhibition technique large-scale serological surveys on man and on domestic and wild animals have become practical. Furthermore, the routine application of the complement-fixation technique for the prompt group identification of isolates has added immensely to the efficiency and interest of work at the Belém laboratory.

A total of 419 strains of arbor viruses was isolated in the Belém area in 1959 from 178 infections. Members of all six serological groups known

to be present in the Belém area are represented in the Year's isolations, but members of groups C and Guama continue to be encountered most frequently, constituting 52.7 and 35.1 per cent respectively, of all infections (Table 1).

We are now having the annual epizootic in horses reported in this region of Pará each year during the early weeks of the rainy season. This is the first year we have had sufficient personnel to send out a group to follow the course of the epizootic. We have now isolated the virus of EEE from horse and from mosquito (Aedes taeniorhynchus), and are studying the birds and wild animals. One of the sick horses from which we did not get EEE yielded a new virus in brain and liver tissue. This same virus also has been isolated this year from wild rodents, sentinel mice, birds, and mosquitoes.

TABLE 1

Arthropod-borne Virus Isolations
by Source and Serological Group
1954-1959

	A	B	C	Guama	Bun	Cal	Misc	Unk	Total
Human	13	23	23	9	4				72
Sentinel monkey	39	3	123	54		1	2	2	224
Sentinel mouse	26	6	218	141			2		393
Other animals	1	3	7	7		1		1	20
Arthropods	9	7	4	2	13	3		1	39
Total	88	42	375	213	17	5	4	4	748

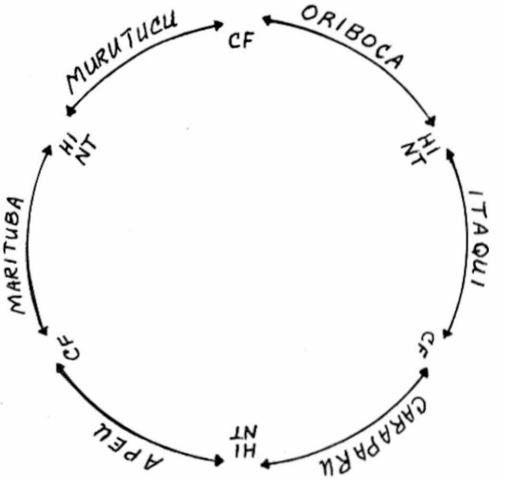
Group:

A	EEE, VEE, Mayaro (H407), Aurá (AR10315), AR13136
B	Bussuquara (AN4116), Ilhéus (H7445), Yellow fever * (H111)
C	Oriboca (AN17), Itaqui (AN12797), Caraparu (AN3994), Apeu (AN848), Marituba (AN15), Murutucu (AN974)
Guama	Catu (H151), Guama (AN277), AR12590
Bunyamwera	Guaroa (H12208), Wyeomyia * (AR278, AR671), Cache Valley * (AR7272), Kairi * (AR8226)
California	Melão (AR8033)
Miscellaneous	Tacaiuma * (AN73), AN7722 *, AN10615 and AN16833
Unknown *	AN8582, AR5058, AN7132, AN7216

* Not isolated in 1959.

FIGURE I

THE RELATIONSHIP OF GROUP C AGENTS BY COMPLEMENT FIXATION, HEMAGGLUTINATION-INHIBITION AND NEUTRALIZATION TESTS.



CF = Complement fixation test

HI = Hemagglutination-inhibition test

NT = Neutralization test

REPORT FROM DR. ROBERT H. KOKERNOT, DIRECTOR,
INSECT-BORNE VIRAL DISEASE RESEARCH UNIT,
THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH,
JOHANNESBURG, UNION OF SOUTH AFRICA

This Unit is jointly-financed by the South African Institute for Medical Research, the Poliomyelitis Research Foundation, the South African Council for Scientific and Industrial Research and The Rockefeller Foundation, with the collaboration of the Union Health Department and the Veterinary Division of the Department of Agriculture. Dr. K. C. Smithburn of The Rockefeller Foundation, able director of the Unit since its establishment in 1953, was unfortunately forced to return permanently to the United States because of ill health early this year. Direction of the Unit was assumed by Dr. R. H. Kokernot. The team of workers continued its coordinated laboratory and field investigations on arthropod-borne viruses.

Field studies on a long-term basis were continued from the field station in northern Natal. Increased emphasis on the isolation of viruses from mosquitoes and birds resulted in the recovery of a somewhat larger number of isolates than in former years. Twenty-one of these came from mosquitoes and three from birds. Not all of the isolates have been finally identified, but it is significant that eighteen of the virus strains from mosquitoes fall into categories that have previously been recognized in South Africa. The added observation that viruses were collected only from mosquito species previously known as viral hosts, makes it seem likely that the work of the past four years in Ndumu has now disclosed the chief types of virus and the chief virus-carrying mosquitoes of the region. It is now planned to test other sorts of creatures as possible harborers of viruses, especially aquatic organisms which frequently are found in close association not only with mosquito larvae but also with water birds and many other terrestrial vertebrates that are susceptible to infection by these viruses.

On the basis of previous work in Portuguese East Africa it was possible to select a few localities in that country where viruses and mosquito vectors were especially prevalent, and a return expedition to these places was made in 1959 in order to attempt the isolation of the viruses themselves. Most of the work was done at Lumbo, a small coastal town in the northern portion of the country. After about three weeks at this station brief collections were made at Namacurra and Nova Lusitania, both of which are further south but still north of the Save River. Three different virus types were recovered from mosquitoes collected by the expedition. Eight strains of one of these was isolated from a species of mosquito, Aedes (Skusea) pembaensis, which has hitherto not been associated with arthropod-borne viruses. The new virus and its associated mosquito present some interesting biological problems which call for a second follow-up trip which is planned for March, 1960. It is of special interest that Aedes pembaensis lays its eggs on crabs and the larvae develop subsequently in the crabs' burrows.

A second virus type was isolated from mosquitoes of the Culex sitiens group at Lumbo. The virus has not yet been identified and it is unfortunately impossible to be specific about the mosquito's identity for this is also a previously unincriminated group in relation to arthropod-borne viruses.

The third isolate, Semliki Forest virus, had not previously been recognized south of Uganda. It was obtained at Namacurra from Aedes (Aedimorphus) argenteopunctatus, similarly a species that was not hitherto associated with arthropod-borne viruses.

A serological and entomological survey of parts of Bechuanaland and the Caprivi Strip was accomplished by an expedition in June-July, 1959. Preliminary results in the laboratory indicate that while viral activity in this region has been widespread, including many viruses known to occur in the Union, the intensity of past viral infection among human beings can be fairly well correlated with presence of surface waters and rainfall zones. Thus areas most closely approaching desert conditions have been the least visited by viruses, presumably because of conditions least favorable for mosquito breeding.

The Unit is collecting aquatic organisms of many sorts and testing them for the presence of arthropod-borne viruses. The basis for this project has at least a triple derivation: 1, the essentially negative results achieved elsewhere by workers who have sought to detect fundamental and permanent viral cycles apart from recognized outbreaks; 2, certain considerations of the observed relationship between viruses and their vertebrate and invertebrate hosts; 3, local findings at Ndumu, Natal, regarding the periodic appearance of numerous virus types in various mosquito hosts.

1. By essentially negative results is meant any sustained effort to demonstrate a mechanism that has not led to a substantial conclusion. For example the discovery of a single infected specimen of Culex tarsalis in winter after years of intensive search hardly solves the question of the year-to-year survival of WEE virus. Other types of negative evidence include: the failure to incriminate migrating birds conclusively as regular long-distance vehicles for viruses; the failure to demonstrate or induce recurrent viremia in immune hosts; the largely negative results with wild rodents in both field and laboratory in attempts to link them strongly with mosquito-borne viruses; the lack of adequately suggestive evidence that parasitic and commensal arthropods in birds' nests play a role in perpetuating viruses found naturally in some avian species.

2. Many findings relating to mosquito-borne viruses suggest that they are better adapted to cold-blooded hosts than to warm-blooded ones. The commonly observed acute reaction in man and domestic animals is in marked contrast to the apparent lack of reaction in infected mosquitoes. Recent work has shown that some of these viruses will multiply following their artificial introduction into a wide range of arthropods other than mosquitoes. The persistence of virus in the blood stream of snakes and of bats under artificial hibernation may have a parallel in the apparent favorability of cold-blooded creatures for these viruses. Infection of fledgling birds by ingestion of artificially infected arthropods indicates a route other than the inoculative one for acquiring viruses.

3. The periodic recurrence of strains of a spectrum of virus types at Ndumu is irregular and sporadic. Human beings and domestic animals show only a low antibody conversion rate even during times when viruses can be recovered from mosquitoes. Isolations from mosquito populations at specific collecting sites have shown so discontinuous a pattern that it has not been

possible to recognize anything resembling an epizootic outbreak. A collecting site may be negative for viruses for a long time, and then suddenly more than one virus type may appear, in more than one species of mosquito, only to be extinguished almost immediately. Viral isolations are affected most commonly in April and May, a time when bird migration from the north is non-existent.

Proposed hypothesis. Some of these viruses may have their natural and fundamental cycles in various aquatic creatures. If so:

1. Mosquito larvae may become infected by the ingestive or inoculative routes (parasitism in the latter instance). This would mean that male mosquitoes as well as females should be found with virus.

2. Larvally infected female mosquitoes could initiate terrestrial viral cycles when feeding on vertebrates. The feeding habits of these initiating species would determine the kinds of vertebrates that become infected. The same mosquito species, as well as other species, would then continue the terrestrial cycle in the same and different kinds of vertebrates as supporting species.

3. Since the infection of mosquito larvae may be only accidental, the initiation of terrestrial cycles would be sporadic. Thus the apparent absence of a particular virus in mosquitoes for one or more years would not mean that the virus was not present in Ndumu aquatic habitats.

4. Viruses may leave the aquatic habitat by other means, particularly following the ingestion of aquatic organisms by birds. However, there is also the possibility of parasitism of birds and mammals by leeches. In addition tadpoles may become infected and following metamorphosis emerge on land as viremic frogs and toads.

5. Some aquatic organisms have annual cycles related to climatic factors leading to periods of breeding, feeding, and suspended activity. During aestivation or cyst formation they may be buried in mud. If they contained virus, we would not be likely to find it during inactive states. Transovarial transmission might occur in water mites.

Conclusions. The approach to the problem is clouded by the fact that we have no evidence for the hypothesis. It is therefore necessary to make a broad survey of the situation. This is being done by routine inoculation of infant mice with:

1. Fully identified male mosquitoes.

2. All types of aquatic creatures identified so far as possible although mostly only to the family.

REPORT FROM MAJOR HERBERT C. BARNETT, CHIEF,
DEPARTMENT OF ENTOMOLOGY, WALTER REED ARMY INSTITUTE OF RESEARCH

Progress Report on Sandfly Fever Studies

A total of 80 lots of sandflies collected in West Pakistan and Iran have been tested to date for viruses pathogenic to suckling mice. Five lots out of 34 lots collected in the Peshawar area of Pakistan have yielded virus isolates. Two of these isolates have been identified as the Sicilian strain of sandfly fever. Five lots collected in Lahore have thus far yielded no isolates. Sixteen lots out of 41 lots collected in the Varamin Plains area of Iran have yielded suckling mouse agents, but none have yet been identified.

These virus strains have been obtained from lots varying in size from 29 to 199 sandflies. All positive lots contained some Phlebotomus papatasi. The isolates were obtained anywhere from the primary inoculation to the fifth blind passage in suckling mice. Four of the isolates have been reisolated from the original sandfly inoculum. Most isolates kill suckling mice in 3 - 4 days after 10 to 12 passages. The five isolates from the Peshawar area have been studied by Major Diercks in hamster kidney tissue culture. Initially, none of them produced cytopathogenic effects, but one strain (one of the Sicilian isolates) became adapted after three passages and produces moderate CPE. Many of the isolates do not develop much more than 1 log titer even after 12 - 14 suckling passages, thus making identification difficult.

Sera collected from three groups of healthy Pakistani soldiers (Bengalis, Punjabis and Peshawaris) have been tested for neutralizing antibodies to the Sicilian strain of sandfly fever. The Bengalis had resided in the Peshawar area for less than one year, the Punjabis for 2 - 4 years and the Peshawaris for life. The results were: Bengalis - 0/31, Punjabis - 5/30, and the Peshawaris - 11/25. These results are compatible with the known distribution and abundance of P. papatasi.

A colony of P. papatasi has been established at the Walter Reed Army Institute of Research. It is now in its fourth laboratory reared generation but is not yet of sufficient size to permit experimental studies.

REPORT FROM DR. JACK R. SCHMIDT,
DEPARTMENT OF VIROLOGY, U.S. NAVAL MEDICAL RESEARCH UNIT No. 3,
CAIRO, EGYPT, U.A.R.

Prior to 1956 this task (formerly NM 52 05 03) was primarily concerned with the definition of the role of arthropod-borne viruses and rickettsia as etiologic agents for human diseases. Significant information was obtained on the epidemiology of such known diseases as West Nile Fever, Q Fever, Sand-fly Fever, Yellow Fever, and Typhus Fever in Egypt and the Sudan. Sindbis virus was discovered and shown to be a widely distributed human disease-causing agent.

Between 1956 and 1958 studies mostly concerned enteroviruses. Polioviruses related to paralytic poliomyelitis were shown to be the usual types with type I predominating, and data confirming the high incidence of the disease in infants was derived. No relationship between Echo and Coxsackie viruses and the occurrence of diarrhea in non-indigenous school children could be defined.

During the past year studies have been in several areas. These are:

a) Equine Encephalomyelitis in collaboration with the Ministry of Agriculture. Known to occur in sporadic epidemics since 1949, but so far undefined as to cause. Tissues from 12 fatal equine cases during spring yielded 3 viruses, 1 West Nile and 2 identical but, so far, unidentified agents. An additional 54 cases occurring during a fall epidemic are still being processed. Blood sera from nearly 500 equines collected in lower and upper Egypt failed to neutralize Eastern, Western, and Venezuelan equine encephalomyelitis viruses, but yielded a high incidence of antibody against West Nile virus ranging from 10% in lower Egypt to 85% in upper Egypt.

b) Sand-Fly fever. Four strains of the Sicilian type of Phlebotomus Fever virus have been isolated from pools of sand flies collected in the Cairo area, over 24,000 sand-flies being processed in the pools. This is the first known isolation of these viruses from wild caught sand-flies and provides confirmatory evidence of the vector role of Phlebotomus. A laboratory reared colony of sand-flies has been established from transmission work. Attempts to demonstrate hemagglutination-inhibiting antibody in 9 types of common mammals and birds of the area failed.

REPORT FROM THE VIRUS RESEARCH CENTRE, POONA, INDIA

Primary attention and utilization of laboratory and field facilities continues to be on Kyasanur Forest Disease. Efforts in 1959 have been hampered by lack of mice. The mouse colony was destroyed because of an infection of unknown etiology and is not expected to be fully restored from newly established breeding stock until late in 1960. Expansion into additional new laboratory facilities completed in July 1959 has also complicated operations at this time. However, the additional space and new equipment and facilities are expected to enhance greatly the capacity of the Virus Research Centre and its personnel in the near future.

The routine isolation from and neutralization testing of field specimens from the KFD area has been transferred to the new Mysore Department of Public Health Virus Diagnostic Laboratory in Shimoga which the Virus Research Centre was instrumental in establishing. Hence, epidemiological information based on routine laboratory examinations continues to accumulate.

The 1959 epidemic season has seen a recurrence of KFD among humans in the known epidemic area and several adjacent villages. The incidence of the disease in man has been approximately of the same order as was seen in

1957 and 1958. Monkeys continue to die in large numbers in the known epidemic area and in the adjacent forests. Confirmation of the presence of KFD virus has been obtained in two new areas, one some 35 miles northwest of the center of the established epidemic area and one some 100 miles to the southeast.

For the first time KFD virus has been isolated from pools of apparently unfed larval ticks indicating transovarial passage of virus, although the significance of this phenomenon for survival of the virus in nature remains to be assessed. Added evidence of the probable pre-eminence of Haemaphysalis spinigera as the vector of KFD virus in the epidemic area has been obtained.

A slowly emerging concept is that KFD may exist in India in a series of scattered pockets, primarily as an infection of various wild animals and birds with only occasional spill over into man under circumstances where his occupation may expose him to tick bite and the ticks of the area concerned feed on a diversity of reservoir hosts and also man.

Work has continued this year studying the effects of the virus of Kyasanur Forest Disease in animals in the laboratory.

The main experiments have been conducted in monkeys (Macaca radiata). In the previous experiments it had been shown that this species of monkey was very susceptible to infection with KFD virus. This monkey is one of the two species involved in the epizootic occurring in the Kyasanur forest disease area. The disease produced in them by the KFD virus seemed to resemble closely the disease in humans. A controlled study was carried out to verify this. The study was particularly concentrated on the effect which the virus had on the haemopoietic system but general clinical details were noted also. It was found that all the monkeys infected with KFD virus showed a marked leucopenia, erythropenia and some thrombocytopenia. The leucopenia, and the thrombocytopenia were similar to that seen in humans. The infected monkeys also showed some depression of bone-marrow activity at the height of the disease. Phagocytosis of the blood elements was noted as occurring in the peripheral blood. This appeared to fit in with the histo-pathological findings of phagocytosis of the peripheral blood elements by the organs of the reticulo-endothelial system. All these findings were studied in relation to the degree and length of the viremia. From a clinical point of view the fact that one of the monkeys relapsed on about the 20th day of illness with central nervous system signs was of great interest. These studies have provided further problems to work on and this work is continuing. The effect of infection with KFD virus in rats (Rattus blanfordi and Rattus rattus wroughtoni) and squirrels (Funambulus) have been studied in addition. These animals are present in the KFD epizootic area and have been shown by routine serological studies to have antibodies against the KFD virus. Infection in the laboratory with the KFD virus indicated that the great majority of the rats become infected and develop antibodies. The Rattus blanfordi appeared more susceptible than the Rattus rattus wroughtoni. However, none of them died as a result of the infection. The squirrels were found to be very susceptible. Very high titres of virus could be demonstrated in their blood at the height of the illness. The majority of the squirrels died of the infection. It was of interest that lice from two of the infected squirrels were

found to have high concentration of KFD virus in them. The squirrels that survived infection showed antibodies against the KFD virus.

Data have been obtained on the sensitivity of tissue culture chick embryo cells to KFD virus. Fairly good correlation has been observed between the isolation of KFD virus from field specimens in infant mice and chick embryo tissue culture. This also has been the case with titration in adult mice and chick embryo tissue culture. The principal difficulty has been the irregularity with which the virus produced cytopathogenic changes, and thus tissue culture fluid must be inoculated into mice to detect the presence of the virus with certainty.

Two strains of KFD virus, P9605 and W372, have been serially passaged in monkey kidney and chick embryo tissue culture. No change has been noticed in their behaviour in tissue culture.

A method has been adapted for plaque production of arbor viruses on monolayers of chick embryo cells. Initial experiments have been satisfactory and additional work is in progress.

As time and facilities permit the plan to continue field surveys to determine the distribution and incidence of arbor viruses in ecologically representative regions of India continues.

A team from the VRC was in the field in Kashmir during the latter part of April and the first three weeks of May. With the fact in mind that viruses closely related to that of Kyasanur forest disease are present in Asia north of the Himalayas, particular emphasis was laid on finding evidence of activity of viruses of the RSSE (tick-borne) virus complex.

Over 700 sera were collected, mostly from humans, but some also from horses, sheep and rodents. The areas in which the bleedings took place were selected carefully to cover as large a representative sample of the indigenous population as possible in places where the terrain was considered as possibly suitable for the survival and activity of the known vectors of the arbor viruses.

It appeared that the vale itself, with the standing water in the paddy fields, might be a suitable habitat for the mosquito vectors of the West Nile - Japanese B complex of viruses. The higher areas where the shepherds graze their flocks might be suitable for the activity of ticks able to transmit the viruses of the RSSE complex. Samples of sera and the arthropods prevalent in each area were collected. Serological studies on the human material showed no activity to the viruses of the RSSE complex and minimal activity against the Japanese B - West Nile - dengue groups.

The entomological material gotten is in the process of being worked up. It was found that Culex tritaeniorhynchus the vector of JBE in Japan is present on the floor of the vale and Ixodes persulcatus and I. ricinus the vectors of various of the tick-borne viruses of Europe was taken on grazing animals on the higher slopes and meadows. Aside from ticks on domestic animals collections were also made on shot and netted birds and trapped small mammals.

In view of the previous evidence for the presence of RSSE complex infection in Saurashtra, a brief visit had been made to Kutiyana, the Gir Forest and several other localities to collect specimens and study ecological conditions in 1958. The results of this field trip confirming the previous findings are summarized in the 1958 annual report.

In September of this year a further visit was made to Kutiyana to attempt to find some common factor in the histories of persons with RSSE/KFD antibodies and to obtain serum specimens from contacts and others who might be involved through the epidemiological leads which might develop. It was possible to get detailed histories from 16 persons whose sera showed evidence of infection on the previous surveys. It was not possible to establish any previous overt illness as being related to the presence of RSSE/KFD antibodies. It was found however that in several instances there were multiple infections in individual families and that unboiled fresh milk is drunk. This is an interesting lead in light of the work of Russian investigators reporting a secondary mode of infection through the ingestion of unboiled goat milk.

A substantial sample of bloods from domestic animals was gotten as well as a further selection of human sera. These are now under investigation.

Investigation of the mosquito-borne virus problems of southeastern India which began with the recognition and long-term investigations of Japanese B encephalitis in Madras in 1955, continues on a gradually increasing scale locally at the Christian Medical College in Vellore. As a consequence of an equipment grant by The Rockefeller Foundation to the Department of Microbiology, a Virology Section is being set up there to work in close collaboration with the VRC field station maintained on the hospital grounds since investigations on Japanese B encephalitis were begun there in 1955. This has involved relocation of the mouse colony and other facilities.

REPORT FROM DR. J. THOMAS GRAYSTON,
U.S. NAVAL MEDICAL RESEARCH UNIT #2, TAIPEI, TAIWAN

Japanese B Encephalitis

Studies during 1959 have been concentrated on completion of serological studies of sera collected during 1958, further serological studies of animals, and isolation attempts from the mosquitoes being collected from three areas of Taiwan.

Sera of 72 adults and 120 seven-year-old resident children of Taiwan were run in the neutralization test with the Japanese B encephalitis (JBE) virus as antigen. Ninety-seven % of the adult serum and 22% of the children serum were positive with a log neutralization index of greater than 1.7. Only 43% of 14 adult sera and 7% of 15 sera from children of Pescadore Island showed neutralizing antibody for JBE. Eight of ten adult sera from Orchid Island were JBE positive in the neutralization test. Serum was collected in May and again in November of 1958 from persons of all ages in Chungho village, a rural section near Taipei. Twenty sera of each age, 1 through 14, were tested in the neutralization test for antibody to JBE. Up to the age of 6, from 5% to 20% of the sera were positive. The per cent of sera positive for the older age groups rose steadily until 80% of the 13 and 14-year-old children showed neutralizing antibody. Paired sera from before and after the 1958 encephalitis season were tested in the HAI antibody test with JBE antigen. Fourteen positive conversions of 359 pairs tested were found. The ages of the children showing conversions range from 5 through 11, and included one 14 year old.

Sera from a variety of species of animals in Taiwan have been tested for JBE neutralizing antibody. Results of these tests are shown in the accompanying table. Of particular interest is the high percentage of neutralizing antibody in pigs and rats.

Isolation of virus from mosquitoes collected, not only in the Taipei area, but also from mid and south island areas, was continued throughout the winter, spring and summer of 1958-59. No viruses were isolated from the end of August of 1958 until June, 1959. One correction in last year's report is to be made. It was stated that all viruses were isolated between July 3 and July 27; however, one additional virus was isolated during the week of August 17 through 23 from a pool of 500 C. tritaeniorhynchus mosquitoes collected in the Taipei area. This virus, although requiring three passages in infant mice before it would infect adult mice, has been shown to be JBE. Mosquitoes collected during the fall, winter and spring seasons failed to yield virus. Two of three mosquito pools of C. tritaeniorhynchus collected during the first week of June, 1959, in the Taipei area, were positive for virus which has not yet been definitely identified. The three subsequent weeks of June yielded two or three viruses from two to five mosquito pools weekly. A total of nine viruses have been isolated in the Taipei area all from C. tritaeniorhynchus mosquitoes. Mosquitoes collected in July of 1959 failed to yield virus. Preliminary information about the human encephalitis season of 1959 shows that it coincided with these June isolations. This is the earliest encephalitis season in Taiwan since reporting was initiated in 1955. Mosquitoes collected at the mid-island station have failed to yield virus so far. Those collected from the southern part of the

Neutralizing antibody for Japanese B Encephalitis virus in the serum of various animals and birds of Taiwan

Species	Time Collected	Number	Per cent Positive
Pig	June-Sept. '58	33	79
Water buffalo	Aug.-Oct. '58	14	14
Goat		7	14
Cat	January, 1959	8	25
Rat	Jan.-May, 1959	10	50
Bandicoot	December, 1958	3	33
Squirrel	December, 1958	15	0
Rabbit	Sept. '58-Jan. '59	31	3
Chicken	Aug. '58-Jan. '59	31	6
Duck	August, 1958	12	0
Bamboo chicken	Jan.-May, 1959	8	13
Water hen	Jan.-May, 1959	12	0
Wild pigeon	January, 1959	7	0
Egret	April, 1958	2	0
Lonchura	Jan.-May, 1959	10 (pools)	20
Sparrow	Feb.-June, 1959	2 (pools)	0
Bittern	May, 1959	3	33
Flying squirrel	May, 1959	1	(pos)

Note: In addition serum from one each of the following species was negative: monkey, mole, deer, civet, falcon, button quail (pool) and bulbul (pool).

island have yielded one virus. This time from the species C. fuscocephalus, the predominant culicine mosquito of that area.

Mosquitoes collected in Okinawa and sent to this laboratory for virus isolation studies have so far failed to yield virus on infant mouse inoculation.

Future Plans

1. Continued study of mosquitoes on the island of Taiwan, both for virus isolations and their bionomics.

2. Further studies of animals and birds as reservoir of encephalitis on Taiwan.

3. Studies of a group of sera collected from a Marine battalion in Okinawa before and after the encephalitis season.

REPORT FROM NAVAL MEDICAL RESEARCH INSTITUTE BETHESDA, MARYLAND

At the present time the Navy has three laboratories having a definite interest in arthropod-borne viruses; they are: NAMRU-2, Taipei, Taiwan; NAMRU-3, Cairo, United Arab Republic; and Naval Medical Research Institute, Bethesda, Maryland. The Disease Vector Control Units at Jacksonville, Florida, and Alameda, California, do not have a direct interest in the problem, but are particularly interested in the ecology of arthropod vectors of disease and their control. Captain Kenneth Knight, MSC, USN, Naval Medical Field Research Laboratory, Camp Lejeune, North Carolina, is carrying out studies on the ecology of mosquitoes and ticks. NAMRU-1, Berkeley, California, is carrying on studies of virus disease - their efforts, which might be of interest to you, concern preservation of tissue cultures and methods of isolation of virus agents; this interest is not particularly aimed at the arbor viruses, but is more general. Preventive Medicine Unit No. 6, at Pearl Harbor, Hawaii, has a small virus laboratory, and has been concerned with outbreaks of encephalitis occurring in the Marines at Okinawa, and is a possible source of isolations. Because of their limited facilities it will be necessary for them to refer such isolates to other laboratories.

Captain H. S. Hurlbut will depart for Taiwan in May for duty with the U.S. Naval Medical Research Unit No. 2, Taipei. In addition to field work on Japanese encephalitis, he will continue his investigation of the arthropod spectra of the arthropod-borne viruses. Virus material which is not excluded from Taiwan may be sent to him after prior arrangement. His address will be NAMRU-2, APO 63, San Francisco, California.

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

I. PHILIPPINE HEMORRHAGIC FEVER.

A. Human and Mosquito (Dengue Group) Viruses. Final identification of the 14 isolates of 1956 from Manila area shows them to be dengue types 2, 3 and 4. Mosquito isolates (*Aedes aegypti* and *Culex tritaeniorhynchus*) were of type 3 only.

II. THAI HEMORRHAGIC FEVER.

A. Human and Aedes aegypti Isolates. This represents a group of 24 viruses not all identified but representing dengue 1 and 2 and chikungunya from man and dengue 2 from Aedes aegypti and another, T-96, not related to any of these. Certain other agents from man are unrelated to dengues or chikungunya.

III. IMMUNOLOGICAL CHARACTERISTICS AND IDENTIFICATION OF VIRUSES FROM HEMORRHAGIC FEVER CASES (PHILIPPINE AND THAI).

A. Dengue Group by C.F. and Neutralization. Complete cross testing is shown of the 4 dengue types. Four type 2 viruses from 4 different parts of the world were cross tested by C.F. only. All these latter proved very similar.

B. Comparison of TH-35 Virus of Thailand with Selected Members of Group A. TH-35 is so closely related to chikungunya it is considered probably identical. It is also closely related to Gulu virus.

IV. THAI HEMORRHAGIC FEVER CASES.

A. Serology and Virus Isolations. From 35 patients from whom paired sera were obtained, the results of virus isolation and serology (CF and NA) against many agents are presented. Practically all had current or recent dengue infection, many had current or recent chikungunya infection also, but few had the latter without the former. A few of the more questionable or mild cases had no evidence of either infection. Other agents, difficult to adapt, have been isolated but not identified. Etiology of the disease syndrome appears to be mixed, but no firm conclusions can be drawn yet.

V. OTHER VIRUSES ISOLATED FROM MOSQUITOES OF THE FAR EAST, 1956, 1958.

A. Viruses at Least Partially Identified. P-886 from the Philippines is closely related to Sindbis, possibly identical.

B. Unidentified Viruses. There are 6 of these, probably all different. Their characteristics are described.

VI. VARIOUS TISSUE CULTURE CELLS TESTED WITH AR-BO VIRUSES, PARTICULARLY FROM THE DENGUE GROUP, AND A FEW MISCELLANEOUS VIRUSES.

Several primary tissue explant cultures not commonly used, i.e., mouse, cat and guinea pig kidney, etc., plus more commonly used ones were tested in search for a system that might substitute for suckling mice. Nothing adequate was found for the dengue group. Some positive findings were found for other viruses.

VII. DEVELOPMENT OF JAPANESE B ENCEPHALITIS VACCINE IN HAMSTER KIDNEY TISSUE CULTURE.

Two types of vaccine production from tissue culture have been under study, (1) inactivated and (2) attenuated. For the inactivated type quantity production methods have indicated 30°C as optimal temperature for virus growth and a 4 hour growth period during the early CPE phase. Hexadecylamine is under

study for concentration, inactivation and adjuvant. In attempts to attenuate, Nakayama strain was abandoned and a mosquito isolate never in mouse passage is undergoing a large variety of passage methods at step-wise decreasing temperatures. A suitable test animal to detect loss of peripheral invasiveness has not been found and appears crucial.

REPORT FROM MAJOR EDWARD L. BUESCHER, CHIEF,
DEPARTMENT OF VIRUS DISEASES, WALTER REED ARMY INSTITUTE
OF RESEARCH, WASHINGTON 12, D.C.

In the evaluation of the potential role of wild birds in the ecology of an arthropod-borne virus in an unstudied area, the serologic survey is the only available simple tool for determining which species are naturally infected and the frequency of exposure. Experience with Japanese Encephalitis (JE) virus infections in herons and egrets in Japan has shown that the frequency of maternal antibody in nestlings, which are more readily captured, accurately reflects the experience of the parent hen. In interpreting results of serologic tests of nestling plasmas, it has been assumed that this maternal antibody was specific for JE virus primarily because no other B group virus likely to induce plurally reacting antibody in birds is known to occur in the temperate zone Far East. However, limited experiments with young herons possessing maternal neutralizing antibody to JE virus showed that some, but not all, resisted challenge infection with this virus. Because these herons are migrants (although the total extent of annual flights for individual birds are unknown), the possibility that such maternal antibody was actually induced by a virus other than JE and endemic in tropical areas had to be considered.

The opportunity to test the resistance of newly hatched birds possessing heterotypic, but reacting antibody, to challenge infection presented in the fall of 1959, when Dr. William F. Scherer indicated that he had a number of domestic hens immune to JE the result of experimental infection. All of these birds possessed neutralizing antibody to JE virus 6-9 months after infection and several to MVE, and WN viruses as well. All were bred to non-immune roosters and select progeny were studied for maternal homotypic and heterotypic antibodies and challenged with JE, MVE and WN viruses. While as yet incomplete, these experiments have shown that the progeny of hens previously infected with JE virus consistently resist infection (as measured by the occurrence of viremia, and HI antibody response) with JE virus during their first week of life (16 of 16 birds from 7 hens protected). On the other hand, with certain exceptions, young birds were generally not resistant to infection with WN (6/69 birds from 9 hens protected) or MVE viruses (3/32 birds from 11 hens protected). Resistance to heterotypic infection, when it occurred did not seem to correlate with the presence of heterotypic antibody in parent hen at laying (the pre-challenge plasmas from protected chicks not yet tested) or with dose size of challenge virus.

Summary:

These preliminary experiments suggest that the protection afforded young chicks by antibody from JE infected hens tends to be specific for this virus. Where protection was afforded against challenge by WN and MVE viruses,

it was apparently independent of the size of challenge dose and in some instances, the pattern of reactivity of parent hen's plasma. Further studies to determine precisely which factor(s) are primarily associated with resistance and susceptibility in these chicks are in progress.

REPORT FROM DR. WILLIAM F. SCHERER
DEPARTMENT OF BACTERIOLOGY, UNIVERSITY OF MINNESOTA
MINNEAPOLIS 14, MINNESOTA

Studies of arthropod-borne viruses in cell and tissue cultures have led to standardization of a plaque assay for Japanese encephalitis virus in chicken embryonic cell cultures with agar. The mechanism of plaque formation is under investigation since without a solid medium above cells, cytonecrosis and plaques do not occur. Plaques have also been formed with Sagiyama virus, but they are small and indistinct, and occur only at lower dilutions than kill suckling mice inoculated intracranially. Calf kidney cells, unlike porcine kidney cells in culture, did not degenerate in the presence of JE virus. When JE virus was passed intracellularly from culture to culture by transfer of human epithelial cells derived originally from liver, virus in one instance increased in cytopathogenicity after 6 serial passages, whereas in another instance, the cell population eventually rid itself of virus. Autointerference was studied with EEE and WEE viruses in L cell cultures, but a zone phenomenon could not be regularly produced.

Studies of Sagiyama virus, a group A arthropod-borne virus recovered from mosquitoes in Japan during 1956, have revealed that it passes a 100 mμ filter, produces goose erythrocyte agglutinins in low titer, and can be assayed and neutralized readily in porcine kidney cell cultures. Virus- and serum-dilution neutralization tests were performed in PK cell cultures and the results compared with those obtained in mice. Infection (neutralizing antibody) by Sagiyama virus near Tokyo has been found in 20% of humans residing on farms where pigs have neutralizing antibody, in 10% of humans in both urban and rural areas, in 67 to 76% of swine, but in essentially no wild herons or egrets. A few herons in mosquito traps during 1956 possessed neutralizing antibody. Sagiyama virus has been recovered from Japanese mosquitoes collected in 1957, the year after its original isolation. Moreover, at the Shinhama heronry, 20-30 miles from the site of original isolation in 1956, a) the virus has now been recovered from mosquitoes, and b) pigs in mosquito traps have been shown to develop neutralizing antibody. Incidental to recoveries of Sagiyama virus from mosquitoes collected during July and August, 1957, were isolations of 33 strains of JE virus.

Attempts to identify another virus (Mag 115) recovered from mosquitoes in Japan during 1956 are under way. Although this virus is resistant to sodium desoxycholate and ethyl ether, Dr. H. Hurlbut has apparently propagated it serially in inoculated mosquitoes, ticks and moth larvae. Another virus (M7/270) different from JE, Sagiyama, and Mag 115 viruses in its behavior in mice has been recovered from "Anopheles sinensis" collected at Sagiyama, Japan, in 1957.

Anamnestic antibody responses occur in monkeys upon intradermal inoculation of small quantities of JE virus, well within the range which a mosquito

might inoculate into man. The mechanism of anamnestic antibody response to mosquito-derived JE virus inoculated intradermally in monkeys, seems to involve merely the antigenic mass inoculated and not depend upon viral multiplication in skin, subcutaneous tissue or blood.

In Japan during 1956 and 1957, it was observed that shooting birds (and bleeding by cardiac puncture) resulted in higher instances of neutralizing substance in plasma than capturing birds by netting (and bleeding by jugular venipuncture). This phenomenon is under study to learn the specificity of the neutralizing substance. Because bile might contaminate blood after shooting or during attempts at cardiac puncture, bile in whole plasma was tested for effect on JE virus despite Theiler's report that "high concentrations of protein" inhibit sodium desoxycholate inactivation of arthropod-borne viruses. Surprisingly chicken bile diluted in chicken plasma (1:100-200) was found to neutralize JE virus, but not to inhibit hemagglutination after acetone precipitation. However, acetone precipitation also reduced the neutralizing capacity of antiserum, and therefore cannot be used to differentiate between neutralization by bile and neutralization by antibody. Studies are currently under way to learn whether the neutralizing substance in plasma of shot birds is specific for one virus, or neutralizes several viruses non-specifically. Also, experiments are being performed to investigate whether tissue-sequestered antibody may be released by the trauma of shooting and cardiac puncture.

REPORT FROM NIH CONFERENCE ON BIRDS AS POSSIBLE DISSEMINATORS OF ARBOR VIRUSES IN NORTH AMERICA

At the Clinical Center in Bethesda on April 4, 1960, the National Institutes of Health convened a conference on North American birds as possible disseminators of arthropod-borne viruses. Co-chairmen were Dr. David E. Davis, Professor of Zoology, Pennsylvania State University, for the ornithologists, and Dr. R. M. Taylor, Department of Epidemiology and Preventive Medicine, Yale University, for the virological epidemiologists. The morning session was devoted to a cursory review of present knowledge of classification, distribution, and ecology of arthropod-borne viruses, particularly in reference to known associations with avian hosts and their ectoparasites. There was also a resume of technical methods for the study of arbor viruses in birds and a global review of arbor virus problems involving birds as potential disseminating hosts, with some attention to various past and present field and laboratory investigations of the problems.

Following discussion and questions, the afternoon session was devoted to presentations by the ornithologists on methods for avian population counts, current knowledge and methods for securing information on migration, the effect of weather and physiological factors on migration of individuals, species, and flocks, and the current status of knowledge about migration of birds from South and Central America into North America.

On the virology and epidemiology panel were Dr. Roy Chamberlain and Dr. Don Stamm of CDC, Dr. William Pond of NIH, Dr. E. L. Buescher of Walter Reed Army Institute of Research, Dr. W. McD. Hammon of the University of

Pittsburgh, and Dr. Telford H. Work of The Rockefeller Foundation. The presentation by the ornithologists included W. W. H. Gunn, Secretary of the Ontario Society of Naturalists, Toronto; Allen Duval, Chief of Migration Studies, U.S. Sports, Fish and Wildlife Service, Patuxent River, Maryland; and Dr. Herbert Friedman, Curator of Birds, U.S. National Museum.

This was the first formal meeting between American ornithologists and virologists concerned with this problem. The importance of closer and more extensive collaboration between these two fields of investigation was emphasized and agreed upon. Some specific proposals for ornithological research of immediate value were discussed and mechanisms for implementation explored. It was decided that a review of the meeting would be prepared and widely distributed through ornithological publications. Dr. David E. Davis will serve as agent for referral of ornithological research proposals pertinent to this problem and as continuing referent for the evolving program of collaborative research.

LIST OF ANNUAL AND OTHER REPORTS RECEIVED

1959 Arthropod-Borne Virus Encephalitis Surveillance Report No. 1
Epidemiology Branch
U.S. Public Health Service
Communicable Disease Center
Atlanta, Georgia

Viral Diseases of the Central Nervous System Surveillance Reports 1, 2, 3
January through October, 1959
California State Department of Public Health
Berkeley, California

Progress Report, September 1, 1955, through March 15, 1959
Ecology of Arthropod-borne Virus Encephalitides and Vertebrate Species
Identification by Blood Studies
School of Public Health
University of California
Berkeley, California

Annual Report (1959) on Studies of Arthropod-borne Viruses
Section of Epidemiology and Preventive Medicine
Yale University School of Medicine
New Haven, Connecticut

Annual Progress Reports, 1 July, 1958, through 30 June, 1959
Department of Virus Diseases
Walter Reed Army Institute of Research
Washington 12, D.C.

Annual Progress Reports, 1 July, 1958, through 30 June, 1959
U.S. Army Medical Research Unit (Malaya)
Institute for Medical Research
Kuala Lumpur, Malaya

1958 Professional Report
U.S. Army 406th Medical General Laboratory
Zama, Japan

Annual Report, 1 March, 1959 - 29 February, 1960
Studies of Arthropod-borne Viruses
Department of Bacteriology
University of Minnesota
Minneapolis, Minnesota

Epidemiologic and Laboratory Studies on Viruses, Principally Arthropod-borne
23 February, 1959, to 22 February, 1960
Department of Epidemiology and Microbiology
Graduate School of Public Health, University of Pittsburgh
Pittsburgh, Pennsylvania

A Review of Activities of the Middle America Research Unit, April, 1958 - September, 1959
Middle America Research Unit
Balboa Heights, Canal Zone

Annual Report, 1959
Belém Virus Laboratory
Belém, Pará, Brazil

1959 Annual Report
Insect-borne Viral Disease Research Unit
Johannesburg, South Africa

Annual Report for the Year 1959
The Rockefeller Foundation Virus Laboratories
66th Street and York Avenue
New York 21, New York

Report of Study Group on Immunological and Haemotological Surveys, December 15-19, 1958
World Health Organization
Geneva, Switzerland

Report of Scientific Group on Research on Birds as Disseminators of Arthropod-borne Viruses, March 9-14, 1959
World Health Organization
Geneva, Switzerland

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