ECOLOGICAL INVESTIGATIONS PROGRAM
ARBOVIRAL DISEASE SECTION
P. O. BOX
FORT COLLINS, COLORADO

ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number Ten October 1964

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EDITORIAL NOTES

Issue number ten of the Arbovirus Infoexchange marks a milestone, not only as the tenth dispatch in little more than four years, but as a uniquely conscientious and expeditious interchange of scientific information on a global scale. Not only are the reports more numerous, giving geographically comprehensive coverage--with the exception of the Asian land mass--on what is really going on in arbovirus research, but it is obvious that the literary quality of the contributions is remarkable, reflecting many factors, not the least of which are the increasing sophistication of our field of science and a more profound appreciation of what each laboratory and investigator's effort means to the whole.

In this issue, we can see the evolution of increased emphasis on investigation of the mammalian host in reports ranging from those on rodents in New Jersey to bats in Africa. While dengue fevers and CNS involvement and mortality from Venezuelan equine encephalitis in man, along with hemorrhagic fever in central South America, continue to be the most serious problems in the New World, hemorrhagic dengue and related Chikungunya occupy an ever-widening area of concern in south and southeastern Asia. The lack of reporting from India fails to give an otherwise balanced perspective to this problem which now looms large enough for the World Health Organization to sponsor a special conference on mosquito transmitted hemorrhagic fevers in Bangkok beginning October 19. This disease struck Calcutta and environs in the latter part of 1963. Here, as in Bangkok, Saigon, Manila, Penang, and Singapore, the etiology is not absolutely clear because both dengue and Chikungunya virus activity has been identified.

Although a summary account of the deliberations of the WHO Regional Arbovirus Laboratory Directors meeting in Geneva July 20-27, will doubtless be included in the next issue of the Infoexchange, it can be stated here that the globally distributed reference laboratories are approaching a pattern of collaborative operation under WHO auspices that is of significant satisfaction to sponsors and collaborators alike, following so many years of persistent encouragement and development.

A list of reagent sera available will soon be distributed as a source from which collaborating arbovirus laboratories can, on definition of need, secure one or another such reagents. These may be difficult or impossible to produce in many laboratories because of practical problems or the exotic nature of the viruses used to produce the material.

The deadline for contributions to issue number eleven of the Arbovirus Information Exchange will be November 30, 1964. Further notification will be sent in October.

Telford H. Work, M.D.
Editor

REPORT FROM DR. R.M. TAYLOR, CHAIRMAN OF THE SUBCOMMITTEE ON EXCHANGE OF INFORMATION ON THE ARTHROPOD-BORNE VIRUSES

Catalogue:

A total of 89 catalogues have now been issued: 36 to institutions and laboratories within the continental United States, one in Canada, and 52 overseas, representing a total of 32 countries.

During the first half of 1964, 252 abstracts and current information were issued on 3 x 5" slips. This makes a total of 2,678 issued to date.

We are now in the process of re-registering on a revised form all viruses now in the catalogue. So far, 45 re-registrations have been received. It is the present plan to commence issuing re-registration cards in October, and it is hoped that they will be completed by the end of the year.

Three new registrations, namely vesicular stomatitis virus (VSV) Indiana strain; Ross Hill (from Australia); and Machupo (the virus responsible for Bolivian hemorrhagic fever) are now being processed and cards will be sent out with the next issue of catalogue material in October.

A Worldwide Non-Primate Animal Virus Cataloguing Program:

Our subcommittee is in touch through Dr. Jacob Traum with the Western Hemisphere Committee on Non-Primate Animal Virus Characterization and it has been tentatively agreed that we will be advised of the report of any "new" viruses which are arthropod-borne and not registered in our catalogue. In return we propose to furnish Dr. Traum, as well as Dr. Brooksby, if he so desires, a copy of our catalogue for cross-reference.

The following circular, accompanied by a registration form, is now being distributed by the Eastern and Western Hemisphere Committees on Non-Primate Animal Virus Characterization:

(SEE NEXT FIVE PAGES)

UNIVERSITY OF CALIFORNIA

NAVAL BIOLOGICAL LABORATORY
VIROLOGY DIVISION, BUILDING T-19
BERKELEY 4, CALIFORNIA

SCHOOL OF PUBLIC HEALTH

Dear Collaborator:

Enclosed are: 1) a statement on the present organization of the Eastern and Western Hemisphere Committees on Non-Primate Animal Viruses Characterization and 2) the Investigator's Data Form agreed upon by both groups.

You may already have submitted, to me, questionnaire forms distributed earlier by the WHO and you may have been among the few investigators advised that the proposed functions of the Western Hemisphere Committee's project had not been fully activated.

The Committees, in the interim, have been occupied principally by the development of a more acceptable questionnaire form, the establishment of procedures to be followed in both hemispheres and the coordination of our activities with other existing committees on virus classification. After extensive correspondence and numerous meetings of the representatives of the three participating agencies, agreement was achieved within the last two months. The Western Hemisphere Committee office is now established and its project fully activated.

Data previously submitted by contributors need not be resubmitted on the new forms. Supplementary information on the same virus, of course, will be welcomed.

Sincerely,

Jacob Traum
Professor of Veterinary Medicine
Emeritus

JT:db

THE EASTERN AND WESTERN HEMISPHERE COMMITTEES ON NON-PRIMATE ANIMAL VIRUS CHARACTERIZATION

A Worldwide Non-Primate Animal Virus Cataloging Program

In recent years accelerated research in virology has created the need for cataloging the information and providing a basis for adequate characterization of numerous viruses which have been isolated. Considerable progress in this direction has been made primarily with certain viruses found in humans by the Entero, Respiratory, and Arbovirus Committees, as well as the Provisional Committee on Nomenclature of Viruses of the International Association of Microbiological Societies.

To date no parallel, concerted program exists for the non-primate animal viruses. Such a program is being initiated under the joint sponsorship of the Western Hemisphere Committee on Animal Virus Characterization of the United States Livestock Sanitary Association, the Eastern Hemisphere Committee on Animal Virus Characterization, and the Veterinary Public Health unit of the World Health Organization. Initially, efforts will be directed towards the gathering, collating and cataloging of data pertinent to the characterization of non-primate animal viruses. The accumulated biological, serological, physico-chemical and structural data will be recorded and catalogued to provide the information in concise form for qualified persons and to serve as a basis for eventual virus classification. In order to assist in more adequate serological identification and comparison of viruses, the Committees will also encourage the production and distribution of reference antigens and antisera. These efforts will be conducted in collaboration with the various other existing virus committees in order to assist in the over-all characterization of viruses and also to obviate, whenever possible, unnecessary duplication.

To carry out this work, your cooperation is needed in supplying information requested on the enclosed questionnaire. This should be done for each distinctly different virus in your laboratory which warrants recording. In submitting such data, it must be kept in mind that this in no way constitutes a publication which, as always, is the responsibility of the individual. Persons using the catalog will be required to obtain permission from the workers who submitted the data before referring to non-published information in public.

Please use a typewriter with black ribbon, if possible, avoiding excessive erasures. This request is made due to the need for photostatic reproduction of the questionnaire. Return with as much data as you can supply to one of the two collection centers: (1) for the Eastern Hemisphere - Dr. J. B. Brooksby, The Research Institute, Pirbright, Surrey, England; (2) for the Western Hemisphere - Dr. Jacob Traum, Building T-19, University of California, Berkeley 4, California, 94720.

NON-PRIMATE ANIMAL VIRUS CHARACTERIZATION PROGRAM

INVESTIGATOR'S DATA SHEET

I. Virus name and/or designation _____

Information from _____ Date _____

Address _____

II. Original source: (Isolation of virus being reported)

A. Isolated by _____

B. Date of collection: day_____ month_____ year_____

C. Animal: (Genus and species) _____ Age _____

Signs of illness and pathology _____

_____

D. Geographic source _____

Specimen collected: (brain, blood, etc.) _____

III. Method of isolation from original source:

	Animal	Avian embryo	Tissue culture
Species or type			
Age			
Type of inoculum (10% brain suspension, whole blood, etc.)			
Route of inoculation			
Signs of infection (nervous tremors, paralysis, etc.) (lesions or changes)			

Has re-isolation been accomplished from original material?

Yes _____ No _____ Not tried _____

Has isolation been accomplished from similar specimens from the same species?

Yes _____ No _____ Not tried _____

IV. Results of inoculation into original host species (clinical, pathologic, immunologic) _____

_____

V. Antigenic Relationship to other viruses:

A. Describe briefly serological tests used (serum neutralizations (SN); Haemagglutination-inhibition (HI); complement-fixation (CF); Other

_____

_____

_____

_____

_____

_____

_____

_____

B. Results:*

Homologous Antigen				Homologous Serum			
Heterologous Sera	SN	HI	CF	Other	Heterologous Virus	SN HI CF	Other

* Record as reciprocal of heterologous titre (HT) over reciprocal of homologous titre (HO) e.g. Nt/Ho

VI. Physico-chemical properties of the virus:

A. Susceptibility to lipid solvents (ether, chloroform, etc.) _____

_____

B. Stability (heat, pH, etc.) _____

_____

C. RNA _____ DNA _____

D. Virus size:

1. Electron microscopy _____
2. Filtration and type utilized _____
3. Other methods _____

_____

E. Fine structure (If detailed electron microscope studies available):

_____

_____

_____

_____

_____

_____

F. Other properties: _____

_____

_____

_____

VII. Other information: _____

VIII. Availability of specimens:
Is virus and/or serum available for distribution: Yes _____ No _____

State restriction on distribution:

IX. Published references:

Please return this form to:

Eastern Hemisphere
Dr. J. B. Brooksby
The Research Institute
Pirbright, Surrey
England

Western Hemisphere
Dr. Jacob Traum
Building T-19
University of California
Berkeley, California 94720

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

Meeting of Directors of WHO Virus Reference Centers:

This meeting will take place in Geneva from July 20-27. The coordination of work among the different WHO Virus Reference Centers and the production and distribution of viral laboratory reagents, especially virus reference strains and reference and working antisera, will be discussed at this meeting.

WHO Seminar on Hemorrhagic Fevers:

A seminar on this subject will take place in Bangkok from October 19 through 27, 1964. The increasingly important problem of the mosquito-transmitted hemorrhagic fevers in countries of the Southeast Asia and Western Pacific regions will be reviewed and discussed. The seminar will be attended by participants from countries in these regions. Dr. W.McD. Hammon, Dr. Scott B. Halstead, and Dr. A. Rudnick have agreed to be WHO consultants at this seminar.

REPORT FROM DR. SUSUMU HOTTA DEPARTMENT OF MICROBIOLOGY, SCHOOL OF MEDICINE, KOBE UNIVERSITY, KOBE, JAPAN

Partial purification of hemagglutinin from type 1 dengue virus and its heterogeneity: (S. Hotta and T. Matumura)

The Mochizuki strain of type 1 dengue virus harvested in brains of 2 to 3-week-old mice was treated by a method slightly modified from the procedures proposed originally by Fukai and Nakamura for JBE virus of mouse brain origin: (1) Infected brains were homogenized with 9 volumes of tris-cystine saline (TCS) (0.1M NaCl in tris buffer, containing 0.01M cystine, pH 8.0) and centrifuged at 8,000xG for 15 minutes; (2) The supernatant was treated with protamine sulfate (at a final concentration of 0.5 mg/ml) at 0°C for 15 minutes and separated from precipitate by centrifugation (2,000xG, 15 minutes); (3) The supernatant was mixed with cold ethanol (at a final concentration of 20%) and kept at 0°C for 30 minutes; (4) The precipitate was resuspended in TCS, that was regarded as partially purified fraction.

HA activity was measured by Clarke-Casals' method using goose erythrocytes; protein contents were determined by Lowry's method. Purification ratios* of the final preparation were in the ranges of 150-200.

Density gradient centrifugation was carried out in sucrose gradients (20-49% in TCS) at 39,000 rpm for 4 hours, using an SW-39 rotor of the Spinco L type centrifuge. The samples thus centrifuged were divided into 12 fractions through a pinhole punched at the bottom of centrifuge tubes.

It was found that HA activity of type 1 dengue virus was separated into two components, i.e., rapid-sedimenting hemagglutinin (Hr) and slow-sedimenting hemagglutin (Hs). Zones represented by Hr were apparently broader than those by Hs. In some cases, HA activity was detected also in zones denser than Hr; it has not yet determined whether such zones might represent the third component or not. The heterogeneity of the hemagglutinin of type 1 dengue virus was evident. Correlation between HA activity and mouse or tissue culture infectivity, as well as electron microscopic pictures are being investigated.

Reference:

Stevens, T.M. & Schlesinger, R.W., Feder. Proc., 22, No. 2, Part I, 674, 1963.

*$\frac{\text{HA/Protein of treated material}}{\text{HA/Protein of original material}}$

REPORT FROM DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF OTAGO, NEW ZEALAND

Fiji Investigations:

The virus strains reported in Information Exchange No. 8, isolated from the blood of four of 60 sick children in Suva have all proved to be strains of Coxsackie A6.

No virus isolations have been made from nearly 2,400 mosquitoes collected within houses in and around Suva, nor has any isolation been made from over four thousand mosquitoes caught on the islands of Viti Levu and Taveuni. Nevertheless, from approximately forty thousand mosquitoes, caught on human bait at the mouth of the Rewa River and

examined in pools of 100, nine strains of virus Coxsackie A6 have been isolated. The mosquitoes, mostly Aedes (Stegomyia) polynesiensis, were caught by the same two mosquito inspectors working together in the forest approximating the village of Nukui. The dates of collection and number of isolates are given below.

Date	Number of pools processed	Number of pools producing virus (all A. polynesiensis)
15 Jan. 1963	36	1
21 Jan. 1963	53	1
22 Jan. 1963	36	1
28 Jan. 1963	50	6

The mosquito inspectors endeavored to catch the mosquitoes as they alighted on their bared arms or trunks before they could probe. Nevertheless, even supposing that a few mosquitoes did bite and imbibe blood, it is unlikely that the mosquito inspectors either individually or consecutively were harboring circulating virus over the two-week period, thus accounting for the repeated isolations of virus. Neither had any illness during the relevant period.

Dengue 1 neutralization tests in mice have been made with 210 sera collected from children under sixteen years of age. Only one serum sample from a boy aged 8 years showed neutralization. This sample, however, was negative on the HI test. In contrast, nine out of sixteen sera from older patients neutralized the virus. Six and five-tenths per cent of the children under sixteen had HI antibodies to Murray Valley encephalitis virus, including two out of 25 in the 0-3 year age group.

New Zealand Investigations:

Mosquito capturing and bird bleeding and banding were continued during 1964, in the Whataroa area of South Westland. A total of over 30,000 mosquitoes of two species was captured using baited stable traps. Over half of the total of mosquitoes captured during the summer months have now been processed and passaged into suckling mice or onto duck-embryo monolayers. The totals of processed mosquitoes are shown in the Table.

Species	Passaged into: Suckling Mice	Duck embryo monolayers
Culex pervigilans	14,349	16,823
Culiseta tonnoiri	2,159	2,378
Total	16,508	19,201

Three agents have been isolated from suckling mice so far.

MP 24: This agent is DCA resistant, kills suckling mice in 2 to 3 days, has no effect on adult mice and does not produce plaques on duck embryo monolayers. The source was a pool of 100 C. tonnoiri.

P181: Kills suckling mice in 3 to 7 days, does not produce plaques on duck embryo monolayers. DCA sensitivity not yet done.

P204: Similar properties to P181. DCA sensitivity not yet done. Both these latter agents came from pools of 100 Culex pervigilans.

Bloods were taken from 91 adult and juvenile black swans captured on Okarito Lagoon. No HI or plaque-neutralizing antibodies have been detected in these sera, but a viral agent was isolated from the blood of a juvenile captured on January 18. This agent (1581) has a long incubation period in suckling mice (11-13 days), does not produce plaques on duck embryo monolayers, and has killed adult mice inoculated intracerebrally but not intraperitoneally. DCA sensitivity tests have been performed but the results have not yet been obtained.

Blood specimens from 204 small birds captured in Japanese mist nets have so far not yielded any virus isolations but processing of these specimens is still in progress. Bloods from four Pukekos (swamp hens) contained no HI or PN antibody and yielded no virus.

Mammal Studies:

Studies on the introduced Australian brush-tailed opossum (Trichosurus vulpecula) have also been undertaken. This marsupial is, except for brief periods of rodent abundance, the most numerous mammal in our study area.

Fifty-one sera from shot possums were examined. No virus was isolated on duck cell monolayers nor, in eight cases, in suckling mice. No sera contained HI antibodies to MVE virus, but five sera gave some neutralization of the group A virus, M78, as shown by plaque reduction. Significant reduction was not found at serum dilutions above 1:2.

Eight possums were studied for susceptibility to MVE and M78 in the laboratory. They were infected with subcutaneous doses of virus between 10^3 to 10^7 p.f.u. The animals were bled daily for six days after infection and no evidence of viremia was found in T.C. or suckling mice inoculated with a 1:10 dilution of blood. No HI antibodies were formed, but neutralizing antibodies developed to high titers. In general, the titers of neutralizing antibody found were in inverse proportion to the dose of infecting virus used.

Susceptibility of Simuliids to Infection by Arboviruses:

Because of the widespread occurrence of the haemophagous simuliid Austrosimulium ungulatum (Tonnoir) in an area where a group A arbovirus occurs in New Zealand, it was decided to investigate the arbovirus vector potential of this insect. In order to determine the ability of these insects to support the growth of arboviruses, wild-caught adult females were injected intra-thoracically with SFV., AMM2354, MRM 39, M78, MVE, SLE, dengue types 1 and 2, Ntaya, and Chittoor viruses. It was found that the 2 Sindbis strains (M78, a New Zealand isolate, and MRM 39 from Australia) and MVE multiplied, the first two maintaining a level of 10^3 - 10^4 p.f.u./insect over a period of 9 days for MRM 39 and 14 days for M78. These were the longest periods tested. Killed insects which were injected as controls were free of virus on the second day. Attempts to transmit to suckling mice on post-inoculation days 6 and 9 for MRM 39 and 6, 9, and 14 for M78 were not successful. Separate assays of head plus salivary glands and bodies of simuliids injected with M78 virus 14 days earlier showed that there was no concentration of virus in the former.

For comparison, a mosquito, Aedes (Halaedes) australis (Erichson) was injected with M78. After an initial drop, the amount of virus increased rapidly to reach a maximum of 10^7 pfu./insect on the 4th day. On this day, one of four mosquitoes biting suckling mice transmitted the virus.

After feeding on suckling mice with viremia levels of 10^8 - 10^9 pfu./ml., A. ungulatum could transmit M78 and SFV mechanically over a period of 24 hours.

It is tentatively concluded from these results that although some of the viruses tested can multiply in the tissues of A. ungulatum, this insect cannot act as a biological vector for them. Mechanical transmission could be possible where vertebrates are circulating large amounts of virus.

REPORT FROM R.L. DOHERTY, H.A. STANDFAST, E.G. WESTAWAY,
B.M. GORMAN, AND B.C. ALLAN
QUEENSLAND INSTITUTE OF MEDICAL RESEARCH
BRISBANE, AUSTRALIA

During the year to June, 1964, weekly mosquito collections were made at several sites near Innisfail, on the north-east coast of Queensland. A total of 37,423 mosquitoes of 56 species were collected. The mosquito catch was correlated with type of trap (Anophelines being caught in light traps, but not in chicken traps), with temperature (light trap catch being closely related to minimum temperature for the period of the year with minimum temperature below 70°F) and rainfall. Two virus strains were isolated from 630 pools of 26,428 mosquitoes. One strain is closely related to and possibly identical with Mapputta virus and one has not yet been identified.

Mitchell River Mission, which has given high isolation rates in wet season studies, was visited in October-November 1963, towards the end of the dry season. Seasonal differences were found in mosquito populations. Thus An. farauti, C. pullus, C. bitaeniorhynchus, and Ae. normanensis made up 24% of wet season light trap collections but only 0.7% in the dry season, and Aedeomyia catasticta and Mansonia crassipes, absent from wet season collections, made up 10.2% of the dry season total. Two hundred sixteen pools of 7,417 mosquitoes from Mitchell River were inoculated into mice. Strains of Kunjin, Kokobera, and Koongol were isolated from mosquitoes collected in March-April, 1963. Three strains of what appears to be a virus not previously recognized (prototype MRM 1178) were isolated from Anophelines collected in October-November 1963.

Five hundred seventy sera from various birds and mammals were tested for HI antibody to the group A viruses Sindbis,

Getah, Bebaru, and Ross River (T48). Sera from horses, cattle, kangaroos, and wallabies reacted more frequently and to higher titer to Ross River virus than to the other three. Reactions apparently to Sindbis were found in domestic fowls and in some cases confirmed by neutralization test. HI reactions to Getah were found in acetone-ether extracted sera from domestic fowls. These sera did not neutralize any of the available group A viruses and following the report by the Disease Ecology Section, Greeley, Colorado, (Information Exchange #9), it was found that kaolin treatment removed the HI response in most of the sera.

Two members of the staff had laboratory infections with Kunjin virus. One had a mild illness with rash and lymphadenopathy, thought clinically to be rubella, and one had mild fever and malaise. Both developed HI, CF, and neutralizing antibodies to Kunjin and the virus was isolated from serum collected from one patient.

Neutralization of four group B arboviruses (MVE, West Nile, Kunjin, and Edge Hill) has been studied by plaque assays in chick embryo fibroblasts, using Porterfield's techniques. Hyperimmune rabbit sera exhibited greater specificity than immune rabbit sera in both the plaque inhibition test and the plaque reduction test (PRT). In the latter test, surviving virus from neutralization mixtures was assayed without dilution. Kunjin antisera neutralized both WN and Kunjin to the same extent. Addition of heat labile factor (as 1:4 final dilution of fresh normal guinea pig serum) enhanced the neutralizing activity of some antisera in the PRT, but the specificity was not improved, and the serum titers remained unchanged. Neutralization indices measured by the intracerebral route in mice were closely similar to those obtained in the PRT.

Several established cell lines have been examined in the search for a plaque assay system with a wider group B virus spectrum. The stable pig kidney (PS) cell line (obtained through Prof. J.A.R. Miles, from Dr. Inoue, Kyoto) has proved of value, in that clear easily counted plaques were produced by all 10 group B viruses tested (see table). Reproducibility was satisfactory with all except dengue type 1 virus, which fluctuated in titer under the present conditions of assay. In some cases, virus titer or plaque characteristics were improved by the incorporation of DEAE-dextran in the agar overlay medium. Cytopathic effects in tube cultures of PS cells were produced by all 10 viruses, although in some cases cultures required observation for 21 days and the titers of dengue virus were low compared to assays in mice.

PLAQUE PRODUCTION BY GROUP B ARBOVIRUSES IN PS CELL MONOLAYERS

Virus	DEAE-Dextran in agar overlay medium					
	200 µg/ml			Nil		
	Titre (PFU/ml) +	Incubation period (days)	Final plaque size (mm)	Relative plaque count (%)	Final plaque size (mm)	Plaque definition
Kunjin	$10^{8.5}$	4	3-4	100	3-4	Clear
West Nile	$10^{9.5}$	3	4-5	100	2-3	Clear
JBE	10^{8}	3-4	5-6	60	5-6	Clear
MVE	10^{8}	4	2½-3½	100	1½-2½	Clear
SLE	10^{8}	5	2½-3	50	2	Clear
Edge Hill	$10^{8.5}$	6	2	50	<1	Clear
Stratford	10^{6}	8	1½	50	1½	Clear
Kokobera	10^{8}	8	1-1½	100	1-1½	Clear
Dengue type 2	$10^{7.5}$	6	2-2½	40	1-1½	Hazy
Dengue type 1	$10^{7.5}$ (max)	9	1-1½	*	*	*

+ Virus pools were 10 or 20% suspensions of infected suckling mouse brain.

* Not tested.

REPORT FROM DEPARTMENT OF MICROBIOLOGY AUSTRALIAN NATIONAL UNIVERSITY CANBERRA, AUSTRALIA

Enhancement of Arbovirus infectivity by Avian antibody (Hawkes):

While titrating neutralizing antibodies to Murray Valley encephalitis virus (MVE) in domestic fowl by the plaque reduction method in chick embryo fibroblast (CEF) monolayers, it was noticed that the antiserum increased, rather than decreased the plaque count. Test counts were up to 12 times the control count (virus plus pre-inoculation serum). A constant virus-serum dilution technique was being used, and although neutralization sometimes occurred at low serum dilutions, enhancement of infectivity always occurred when virus was mixed with more dilute sera. Further investigation indicated the MVE antisera produced in mice, rabbits, guinea pigs, and pigs did not enhance MVE infectivity when assayed on CEF monolayers. MVE fowl antisera demonstrated the effect on CEF monolayers and the chorioallantoic membrane of the chick embryo, but not in suckling mice or in a line of pig kidney cells (Pk-2a). MVE antisera from pigeons and geese enhance MVE virus, and several sera collected from birds in serum surveys have also demonstrated the effect.

West Nile virus and Japanese encephalitis virus in Group B, and Getah virus in group A exhibit enhancement, but Semliki Forest virus, Sindbis virus, and Ross River virus within Group A do not. Cross enhancement occurs between members of group B, but not between viruses from different groups (e.g. MVE and Getah). Detailed results will appear in the August 1964 issue of Australian Journal of Experimental Biology and Medical Science.

Serological survey of animal sera from the Sepik District, New Guinea:

Animal sera collected in the Sepik District of New Guinea were tested for HI antibodies against Sindbis, Semliki Forest, and Ross River (T48) viruses representing the Group A arboviruses, Murray Valley encephalitis (MVE) virus representing the Group B arboviruses, and Wongal (MRM 168) virus representing the Koongol-Wongal group.

HI antibody to MVE is fairly common right through the animal kingdom and the incidence of reactors is not

significantly different with changes in ecology. There are at least two Group A arboviruses present; one similar to, if not identical with, Sindbis virus, and the other antigenically related to Ross River virus. Sindbis antibody activity is observed only in the bird population especially domestic fowls. Collections previous to February of this year have yielded no reactors to Sindbis antigen. Serological survey during the same period of this year indicates a spilling over of Sindbis activity into the human population.

Out of nearly a thousand animal sera tested to date, none has been found to react with Wongal antigen.

The use of liquid nitrogen to store and transport field specimens (Marshall):

During a recent field trip to the Sepik district of New Guinea, portable "Linde" LR-10-5 liquid nitrogen storage containers were used for the first time. These were charged with liquid nitrogen at Canberra and shuttled to and from the study area once a week. The containers were carried with the collecting teams, and mosquitoes and tissues stored as soon as they were sorted.

Under our conditions of approximately 4,000 air miles, 80 miles of jeep transportation over primitive tracks, 100 canoe miles, and opening once a day in tropical conditions, the charge of liquid nitrogen lasts for about 14 days. The fully charged container weighs about 35 lbs.

To avoid cracking of sealed glass ampoules, small screw cap vials are being used. It is thought that the occasional leaking of these vials is probably unimportant, and limited laboratory experiments have indicated that mosquitoes infected with Semliki Forest virus and directly exposed to liquid nitrogen for 8 days show no greater drop in titer than controls held in a Revco.

Seventy-five vials (1-7/8 x 1/2") can be stored in one container. Each vial comfortably holds 300 mosquitoes in groups of 100 separated by cotton pledgets. Smaller vials would be more appropriate for tissue specimens.

Transportation of liquid nitrogen in passenger aircraft is specifically covered by the International Air Transportation Association (IATA) Restricted Articles Regulations Section IV, Article 1275, and Packaging Note No. 808. These regulations are available for examination at the office of any IATA member.

Briefly, the regulations state that up to 50 litres of liquid nitrogen can be carried in passenger aircraft provided the nitrogen is not under pressure and that it is in a prescribed container. The Linde LR-10-5 conforms to these requirements. The container should be labelled "Nitrogen-liquid. Non-pressurized", and at 120° intervals around the cylinder, with an arrow indicating the upright position and the words "Keep Upright, Do Not Drop". Although not required by regulation, we have further labelled our containers with the statement "Packaging and volume conforms with IATA Restricted Articles Regulations Section IV Article 1275 and Packaging Note 808 for carriage on passenger aircraft". All these labels are painted on the containers, permanently.

Section I of the IATA regulations requires that each consignment be accompanied by a shipper's certificate as to contents. Although we have had appropriate forms printed and submit them with each consignment, in practice we find that they are not demanded. Specimen form is reproduced below.

Shipper's Certification for Restricted Articles
(IATA Regulations Section I)

This is to certify that the contents of this consignment are properly described by name and are packed according to the IATA Restricted Articles Regulations (Section IV, Article 1275. Packaging Note 808 (a)) and to all applicable carrier and government regulations. This consignment is within the limitation of 50 litres prescribed for passenger carrying aircraft.

Class ORA.C. NITROGEN, LIQUID NON-PRESSURIZED

.....LITRES, WHEN PACKED

Per:

Date:

REPORT FROM DR. LIM KOK ANN, DEPARTMENT OF BACTERIOLOGY
UNIVERSITY OF SINGAPORE, SINGAPORE 3

Hemorrhagic Fever in Singapore:

Hemorrhagic fever was first reported in Singapore in 1960. It differed from the Bangkok-Manila syndrome in several aspects. The disease in Singapore was milder; it tended to occur in older children and young adults, and there were no known instances of shock or death. Two dengue viruses (one type 1 and one type 2) were isolated from the acute phase sera of these patients. In 1961 and 1962, hemorrhagic fever continued to occur, with more isolations of dengue viruses from patients' sera (Bull. Wld. Hlth. Org. 30, 227, 1964). Beginning in late 1962, the more severe form of hemorrhagic fever began to occur in younger children, and each year since there have been a number of deaths and also a number of cases who went into shock but recovered. Thirteen viruses were isolated from the patients' sera in 1961 and a few have been identified as dengue viruses. One virus, which has not yet been identified, was isolated from a patient who had shock and recovered. Serological findings in patients' sera in 1963 showed that as in previous years only dengue viruses were involved, and in no instance was Chikungunya antibody (by CF test) detected in the sera.

In 1964, from January to June, 259 specimens of acute sera from patients were received by the laboratory for HF study. They were mostly from children and young adults. The predominant clinical findings included high temperature, hepatomegaly, various rashes, bleeding into the skin and GI tract, thrombocytopenia, and leukopenia. There were 11 deaths in this group of cases, mostly in children under the age of 10 years, and four cases of shock followed by recovery. Significant rise or very high titres of CF antibodies to the four types of dengue virus were demonstrated in many of the patients' sera tested to date. Clinical, virological, and epidemiological aspects of the disease are being studied by the Department of Pediatrics, Dr. Robert Goldsmith of the Hooper Foundation, University of California Medical Center, and Dr. Chan of our department.

Dengue Virus Isolation Procedure:

Undiluted human sera were inoculated into 1-day-old white mice both subcutaneously (in the neck region) and intracerebrally. This has been the standard isolation procedure

used since 1960. In almost all instances from which dengue viruses were isolated, a proportion of the inoculated mice were either sick, paralyzed, or dead in the first passage (incubation periods ranging from 7 to 14 days). From January through June, 1964, 15 viruses were isolated in this manner. Numerous serum specimens have also been put through two blind passages at about 10-day-intervals, but without virus recovery, except in one case when a virus was recovered in the third passage. The diluent used for brain passages is 0.75% BPA at pH 9.

REPORT FROM MAJOR SCOTT B. HALSTEAD AND DR. CHARAS YAMARAT VIRUS DEPARTMENT, SEATO MEDICAL RESEARCH LABORATORY, AND SCHOOL OF PUBLIC HEALTH, BANGKOK, THAILAND

Thai Hemorrhagic Fever Studies

Epidemic of 1964:

Hemorrhagic fever is again epidemic in Thailand. To the end of June, 2080 hospitalized cases in Bangkok and Thonburi hospitals with 143 deaths were attributed to this disease (see Table 1). As in previous years, cases are almost entirely restricted to children 14 years and younger. From unconfirmed reports, it appears that disease is occurring very widely in Thailand. Particularly involved are towns of Northeast Thailand, a region of the country almost entirely unaffected in previous years. Despite the apparent absence of hemorrhagic fever in Northeast Thailand before 1964, serologic surveys of inhabitants of the area have shown that antibody, presumably of dengue origin, has a similar age distribution to that found in residents of Bangkok. It is not clear whether this endemic dengue of past years was less virulent or merely occurred at frequency levels below the threshold of observability.

Table 1. Month cases and deaths due to Thai hemorrhagic fever reported in Bangkok and Thonburi, Thailand. January to June, 1964.

Month	Hospitalizations	Deaths
January	103	8
February	126	4
March	210	11
April	298	23
May	417	37
June	926	60
TOTAL	2080	143

In response to recurrent epidemic hemorrhagic fever, the Ministry of Health of Thailand has inaugurated a pilot Aedes aegypti control project to test control methods which may later be adopted for the entire city. Since DDT is being used, the problem of emergence of DDT resistant Aedes aegypti is being monitored both by the Ministry of Health and Entomology Department of the SEATO Medical Research Laboratory.

In addition to the pilot control project, a DDT fogging and residual spray campaign is being waged by the Municipal Health Department of Bangkok. This program concentrates upon spraying homes of hemorrhagic fever patients and surrounding houses. Previous house by house epidemiologic studies of Thai hemorrhagic fever have shown that cases tend to occur in focal areas with multiple cases occurring frequently in the same family dwelling unit.

On July 20, the US Army-SEATO Medical Research Laboratory, in collaboration with the Royal Thai Ministry of Health and the Royal Thai Army Medical Service opened a Center for Clinical Study of Thai hemorrhagic fever. With a modern research biochemical laboratory to augment the care of the patient with Thai hemorrhagic fever, it is hoped a more comprehensive pathophysiologic understanding of the disease will be achieved. This 40-bed hospital ward is staffed by eight Thai pediatricians and one American pediatrician and supported by the laboratory facilities of the SEATO Clinical Research Center.

Laboratory Studies:

Dengue Virus in Tissue Culture. For the past six months dengue virus plaques under agar have been produced successfully using a calf serum adapted strain of LLC MK2 cells. These cells had previously proved to be unusually susceptible to cytopathic changes when infected with dengue viruses. Results of these early studies have been published (1). Recent studies have shown that detectable but incomplete cytopathic effect is noted to high titers with all high mouse passage dengue virus prototypes. Following inoculation of dengue viruses

of low mouse passage CPE is more complete and the ratio of mouse titer to CPE titer nearer unity than with high mouse passage dengues.

This line of cells has been used for producing dengue plaques under an overlay of special Nobel agar (Difco) containing 0.01% DEAE dextran. A second agar overlay containing 1:10,000 neutral red is added on day 7 and plaques read 1-2 days later. Plaque sizes range from 2 to 4 mm, with some morphologic variations between virus types. Composition of growth and maintenance medium is shown in Table 2.

A pH of 8.0 in overlay medium and during the period of virus absorption has been found to be critical for producing optimal plaque size and plaque count. Plaque counts generally are within 1 log of suckling mouse or BS-C-1 titers and are highly reproducible. In preliminary experiments, plaque counts are linear when tenfold dilutions are made. This cell line has maintained uniform growth characteristics and morphology during over one and a half years of continuous cultivation in this laboratory. In our hands, MK2 cells provide a reliable and sensitive plaque assay method for dengue viruses of multiple serotypes.

Limited copies of the Annual Report of the SEATO Medical Research Laboratory are available on request.

Reference:

(1) Halstead, S.B., Sukhavachana, P., and Nisalak, A. Assay of mouse adapted dengue viruses in mammalian cell cultures by an interference method. Proc. Soc. Exp. Biol. Med., Vol. 115, 1062-1068, April, 1964.

Table 2. Outgrowth and overlay medium for dengue virus plaque production in LLC MK2 cells.

Outgrowth medium

20.0 ml	calf serum
1.0 ml	glutamine, 5%
15.0 ml	tryptose phosphate broth
1.0 ml	yeastolate 10%
0.7 ml	Na HCO_3 7%

Add M 199 to make 100.0 ml
penicillin 300 units/ml
streptomycin 300 µg/ml

Overlay medium #1

2.0 ml	amino acids, Hela (Difco) 100x
2.0 ml	vitamins, Eagle L (Difco) 100x
2.0 ml	glutamine 5%
20.0 ml	calf serum
6.0 ml	Na HCO_3, 4.0%

Make to 100.0 ml with 2x Hanks BSS. Mix with equal amount 2% special Nobel Agar, Difco, containing 0.01% DEAE dextran.

Overlay #2 contains Eagle's medium plus neutral red 1:10,000 final concentration.

REPORT FROM DRS. A. CHIPPEAUX AND CL. CHIPPEAUX-HYPPOLITE OF THE PASTEUR INSTITUTE, BANGUI, CENTRAL AFRICAN REPUBLIC

(Translated by W.L. Armstrong, Library of the Communicable Disease Center, Atlanta, Georgia)

In 1961, the laboratories were opened, the personnel assembled, the equipment installed, and the technics were tested and the contacts made. During 1962 and 1963, a systematic program of investigation was initiated.

Immunological Investigations:

Preliminary investigations were conducted on 217 human sera collected within each of the five zones of the Central African Republic, selected according to various geographical and ecological factors. We have utilized the hemagglutination inhibition test (HI) according to the technic of Clarke and Casals. Some of the tests were conducted at the Pasteur Institute of Bangui. The studies for arboviruses were completed by us at the laboratory of the Pasteur Institute in Paris. Sera having an inhibition of 1/20 or over were considered positive. The results appear in Table I. Following analysis of these results, investigations were resumed in two regions: 1) Lobaye in the great equatorial forest/jungle; and 2) Bovar on the northwestern plateau.

A survey was also begun utilizing sera from 263 children from Bangui. The results were analyzed by age group in order to learn: 1) the immunological status against the yellow fever virus (Mass vaccination required by law was carried out regularly until 1960. It was discontinued after that year.); and 2) the age of spontaneous (natural) acquisition of antibodies to three antigens of group B other than yellow fever: West Nile, Zika, and Uganda S. Neutralization tests in mice are now being carried out to confirm the HI test results.

Paired sera from 60 patients from Bangui and Plateau de Bovar afflicted with various diseases producing the clinical picture of an arbovirosis have been collected. They are being studied by means of the HI test. Results of these tests will be reported later.

Various animal sera are being HI tested with the assortment of antigens available at the Pasteur Institute of Bangui. Monkey sera have been extracted with kaolin, the sera of the other animals with acetone. Results of these tests appear in Table IV.

The examination of the sera of monkeys and lemurs has been supplemented whenever possible by a sero-neutralization test in mice against the yellow fever virus.

Four hundred sera from bovines/Bovidae have been collected in the main centers of animal husbandry (northwest, west, central (Bangui).

Investigation of potential refervoirs of arboviruses has been initiated by inventory of the fauna and testing of the susceptibility of various species of rodents to yellow fever virus (neurotropic French strain) and West Nile virus (strain B 956 of the East African Virus Research Institute). Isolation attempts have also been made. These results will be presented in a later report.

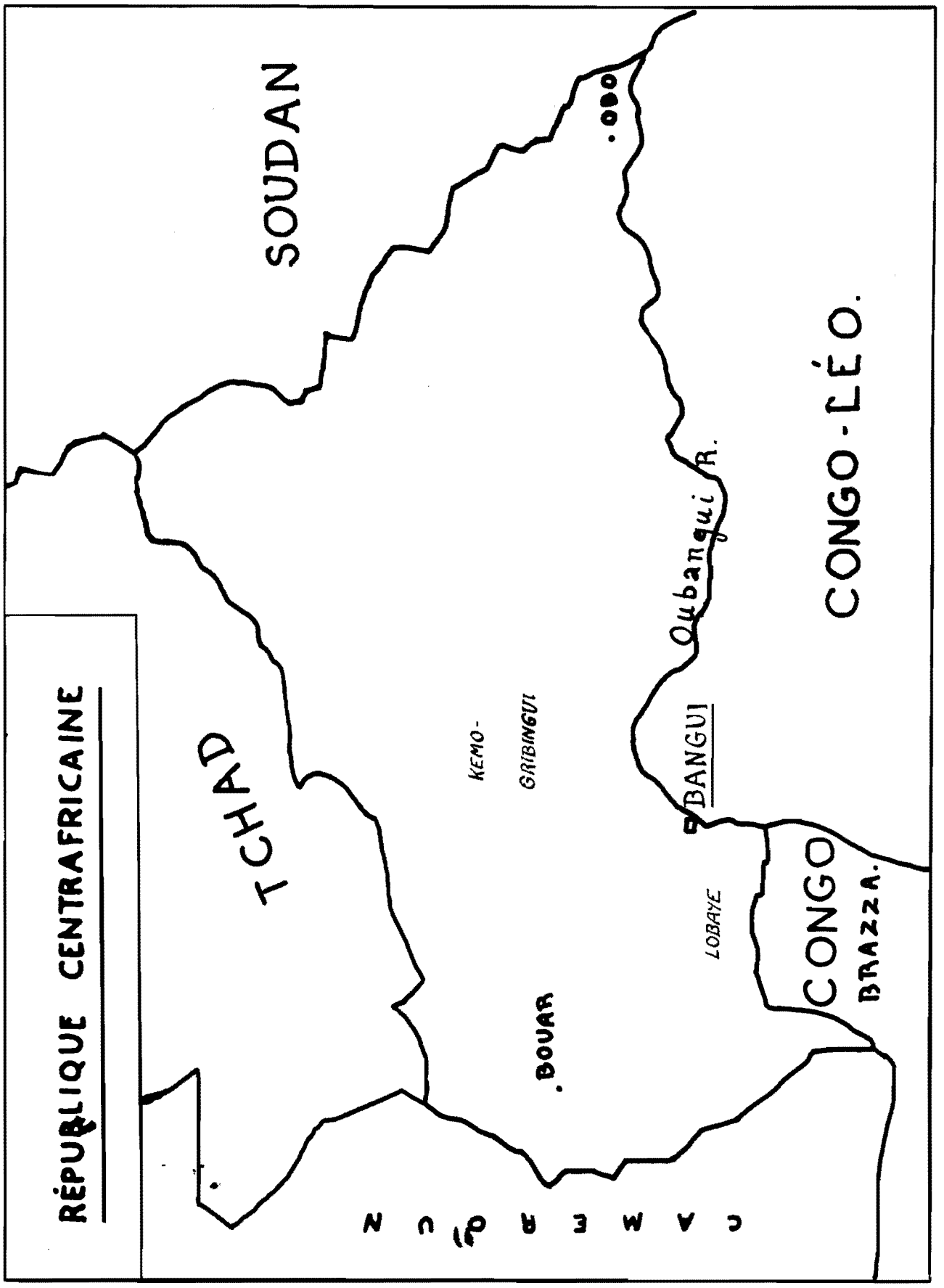

RÉPUBLIQUE CENTRAFRICAINE
SOUDAN
TCHAD
CAMEROUN
CONGO-LÉO.
CONGO
BRAZZA.
Oubangui R.
BANGUI
BOUAR
KEMO-
GRIBINGUI
LOBAYE

TABLE I

PERCENTAGE OF POSITIVES BY HI TEST WITH ARBOVIRUS ANTIGENS WITH FIRST SERA COLLECTED IN DIFFERENT GEOGRAPHICAL AREAS OF THE CENTRAL AFRICAN REPUBLIC

GEOGRAPHICAL FEATURES	ANTIGENS										
	CHIK.	SEMLIKI	SINDBIS	DNG.I	MVE	W.NILE	NTAYA	YELLOW FEVER	UGS	ZIKA	BUN.
Great equatorial forest/jungle (Lobaye) on 41 sera.	46	27	27	10	17	24	46	32	NT	34	36
Wooded savannah of the extreme east (Obo) on 36 sera.	92	58	36	28	33	28	39	56	NT	69	53
Northwestern plateau (Bovar) on 40 sera.	80	20	10	7	NT	25	30	80	NT	25	15
Wooded valley of the Ubangi, on 84 sera.	49	32	6	10	NT	37	61	57	20	65	17
Crossing zone between the Ubangi and Chari basins (Kemo-Gribingui) on 16 sera.	81	12	0	0	NT	12	31	87	NT	12	0

Note: NT indicates that the test has not been conducted upon the sera of that category with the antigen in question.

TABLE II

ANALYSIS BY AGE OF HI REACTIONS TO ARBOVIRUS ANTIGENS

	ANTIGENS						
	CHIKUNGUNYA	SINDBIS	WEST NILE	YELLOW FEVER	UGANDA S.	ZIKA	BUNYAMWERA
Lobaye, in the great equatorial jungle							
On 251 sera from persons aged 1 to 20 years	15.6%	24.5%	15.5%	35%	12%	26.4%	11.5%
On 149 sera from those more than 20 years of age	51%	33.5%	54%	59%	47%	50%	20%
Bovar, on the north-western plateau							
On 76 sera from persons aged 1 to 20 years	38%	3%	5%	29%	1%	6.5%	6.5%
On 24 sera from persons more than 20 years of age	58%	0	41%	67%	17%	29%	0

TABLE III

AGE GROUP ANALYSIS OF ARBOVIRUS HI REACTIONS IN RESIDENTS OF BANGUI

AGES	NO. OF SERA	ANTIGENS			
		DAKAR	WEST NILE	UGANDA S	ZIKA
1-5 years	25	0	8%	4%	4%
6-10 years	139	21.5%	13%	4%	15%
11-15 years	78	42%	24%	11%	32%
16-20 years	21	66%	52%	5%	25%

TABLE IV

POSITIVE HI REACTIONS WITH ANIMAL SERA

GENUS	NO.	ANTIGENS						
		CHIKUNGUNYA	SINDBIS	WEST NILE	YELLOW FEVER	UGANDA S	ZIKA	BUNYAM.
Papio doguera	23	0	0	0	0	0	0	0
Cercopithecus	19	1(1/80)	1(1/20)	Sup.a 1(1/40)	1(1/20)	0	3(1/40) (1/40) (1/80)	1(1/20)
Erythrocebus patas	4	0	0	0	0	0	0	0
Cercocebus	4	1(1/40)	0	1(1/40)	0	0	1(1/40)	0
Colobus	2	0	0	0	0	0	0	0
Pantroglodytes	2	0	0	0	0	0	0	0
Perodictus potto	1	0	0	0	0	0	0	0
Galago	1	0	0	0	0	0	1(1/80)	0
Anomalurus	1	1/20	0	0	0	1/20	1/80	0

REPORT FROM DR. OTTIS R. CAUSEY
ARBOVIRUS RESEARCH PROGRAM
UNIVERSITY OF IBADAN, NIGERIA

The new arbovirus research program at the University of Ibadan in Nigeria is in the process of installation in a building approaching completion by the side of the Department of Preventive and Social Medicine on the grounds of the University College Hospital in Ibadan. The laboratories are not finished, but the developing mouse colony has been allowed to occupy the animal quarters while electricians, painters, plumbers, and carpenters continued operations. The colony is scheduled to reach full production by early August when the progeny of the original stock is expected to be yielding about 70 to 80 litters per day for inoculation and sentinel duty. Through the generous cooperation of the WACMAR Laboratory at Yaba, Lagos, 4000 young Swiss mice were made available in two installments to start the colony. The newborn mice are sexed and the males in excess of future colony requirements are used for virus isolation attempts. Between June 8 and July 15, seventy samples were tested, including human sera, a few arthropod pools, sentinels, and lizards. Agent H35 was obtained from a febrile child, bled at the Adeoyo Hospital in a special children's clinic maintained for the Department of Preventive Medicine. The virus is filtrable through a Seitz EK pad, and is DCA sensitive. Infant mice die with an average survival time of one to two days following i.c. or i.p. inoculation and 10-day-old mice develop paralysis of the rear extremities on about the seventh day after inoculation. Weanlings and adults show no signs of illness.

At the time this report is written, twelve days after inoculation of the original specimen, no serological identification has been attempted. But with homologous serum now at hand, material will be submitted to a qualified center for identification. When the necessary refrigeration equipment is installed, it is expected to maintain a battery of diagnostic sera adequate at least for preliminary typing of isolates.

REPORT FROM ARBOVIRUS RESEARCH UNIT SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH JOHANNESBURG

Chikungunya:

As described in recent publications and reports from this unit, evidence is accumulating that wild primates are natural hosts to chikungunya virus. Investigations in this direction are being pursued at our field study area in Natal where a wild primate trapping project has been started in the riverine forest along the Usutu River. Bloods are being collected from monkeys, which are released after marking. From December 1963 to May 1964, 18 bloods were collected, including five samples from recaptured animals. Six samples were positive to chikungunya virus. The possibility of Semliki Forest infections was eliminated by including this virus in the tests.

There were no conversions to chikungunya virus among the recaptured animals, but tests with West Nile virus showed that two monkeys had recently been infected with this virus. One of the monkeys positive to chikungunya virus was estimated to be 2-3 years old. This evidence of recent chikungunya activity is interesting in view of our failure to isolate this virus from the thousands of mosquitoes collected in the vicinity over the past nine years.

Both epidemics of chikungunya known to have occurred in Southern Africa have been associated with large perennial rivers. This prompted us to start the monkey trapping in the riverine forests along the Usutu. Observations in this forest revealed that Mansonia africana is extremely prevalent there and did occur in the canopy. The vector potential of this species has recently been investigated.

Three separate attempts were made with adult mosquitoes collected in Natal and brought to the laboratory in Johannesburg. Mortality among the mosquitoes was high and they fed poorly. Attempted infection in each instance was by feeding on a viremic monkey. Transmissions were attempted by subsequent feeding of infected mosquitoes on infant mice. After the transmission attempts, the infectivity rates were determined by inoculation of individual mosquitoes into infant mice. The results are shown in the accompanying table. From the results, it appears that M. africana possesses a medium degree of vector capability, and that at least 5.0 logs of viremia

are necessary to infect this mosquito. However, this level of viremia appears to occur frequently in the vervet monkey and baboon (a viremia of 7.0 has been recorded in a monkey and 6.4 in a baboon).

In view of the habits of this mosquito and its vector capability, it appears to be a likely candidate for implication in wild transmission cycles of chikungunya virus in Southern Africa.

Attempted Transmission of Chikungunya Virus by Mansonia africana

Experiment No.	Infective dose (logs)	Post Infective Day Transmission Attempted	Result	Infectivity of Mosquitoes: Infective/Tested
1	3.3	12	Neg	0/6
2	5.3	14	Pos	3/9
3	4.7	9	Neg	0/6
		14	Neg	

Sindbis and West Nile:

For the third consecutive summer, intensive field studies were made at the Olifantsvlei study area near Johannesburg. From approximately 100,000 mosquitoes collected during the 1963-64 summer and inoculated into mice, 18 strains of virus were isolated. These were identified as 9 strains of Sindbis, 8 of West Nile, with 1 strain, so far, unidentified. This is the third consecutive summer in which Sindbis and West Nile viruses have been isolated at Olifantsvlei. Seventeen of last summer's isolates come from 15,266 Culex univittatus, an infectivity rate similar to last year. It would seem that this mosquito is the main vector of Sindbis and West Nile viruses on theSouth African highveld; a habitat with severe winters during which all mosquito activity ceases. Studies on the feeding preferences of mosquitoes at Olifantsvlei showed C. univittatus to possess a low feeding rate for man. In fact, the evidence indicated that this mosquito did not feed on man. This was unexpected in view of observations on this mosquito elsewhere in Africa. On the highveld, it seems likely that other species, possibly Culex theileri, Aedes lineatopennis, A. caballus, and, in urban localities, Culex quinquefasciatus, are concerned in human infections. The absence of epidemics would suggest that the connecting link between wild cycles and

man is somewhat inefficient as a not-insignificant amount of transmission by both viruses occurs in a domestic environment, i.e., in the yards of human dwellings.

A few human infections by both Sindbis and West Nile viruses were again encountered this past summer on the highveld. West Nile virus was isolated from one case while the remaining West Nile infections and all of those due to Sindbis virus were diagnosed serologically.

REPORT FROM THE VETERINARY RESEARCH INSTITUTE ONDERSTEPPORT, SOUTH AFRICA

The successful propagation of a type 3 strain of horse-sickness virus in monolayer cultures of chicken embryo fibroblasts is described. The nutrient medium consisted of Hanks' balanced salt solution to which 0.5 per cent lactalbumin hydrolysate, 10 per cent bovine amniotic fluid and 2 per cent bovine serum were added.

Daily titrations of tissue culture material in day-old mice confirmed that virus multiplication took place in spite of the absence of visible cytopathic effects. The specificity of the mortality in mice was controlled by means of a complement fixation test.

The above virus strain was carried successfully through six serial passages and subsequently a type 4 strain of horsesickness virus was similarly adapted to multiply in chicken embryo fibroblasts.

Reference: Erasmus, B.J. Cultivation of Horsesickness Virus in Tissue Culture. (Correspondence) Nature 1963, Nov. 16, V 200, 716 (7 refs).

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE ENTEBBE, UGANDA

Yellow Fever:

Blood and CSF were received from New Mulago Hospital in May, 1964, from a fatal case with a typical clinical history of yellow fever. Yellow fever virus was isolated and reisolated from both specimens, and confirmed by cross-neutralization tests with the French Neurotropic strain. HA tests showed the isolate to be closer

antigenically to a recent yellow fever strain from Ethiopia than to French Neurotropic.

The patient, an African male, lived 20 miles northeast of Kampala, Uganda, and had not left his home area for more than a month before his illness. His house is on a hilltop, and a strip of forest partially encircles the base of the hill, while a large forest reserve lies a quarter-mile away from his house as the mosquito flies. Mosquito catches were instituted in these two areas of forest, and have so far resulted in the isolation of at least two strains of yellow fever virus from Aedes africanus caught in June 1964, but none from the other species of culicines caught.

This is the most easterly case to have been confirmed in Uganda, the other two recorded deaths having been in the Impenetrable Forest in Bwamba District, 170 miles west of Kampala, where yellow fever is known to be endemic.

Bat Viruses:

Three more Group B viruses have been isolated from the salivary glands of 479 Tadarida spp. caught in Uganda, bringing the total of isolates to 9. This gives a crude infection rate of about 1:50 bats, but if the bats taken from colonies which have not yielded virus are excluded, the rate for virus positive colonies is much higher.

REPORT FROM DR. P. BRES, CHIEF OF LABORATORY, AND DR. L. CHAMBON, DIRECTOR, PASTEUR INSTITUTE DAKAR, REPUBLIC OF SENEGAL

Our first study of bats, naturally infected with arboviruses in Senegal, is now completed (see Info Exchange No. 9). We processed 3,115 insectivorous bats from July 1962 to December 1963.

Forty-two strains of Dakar 249 (Group B new?) and 2 of Chikungunya were isolated. At first sight, such a high figure seemed suspicious to us, but then we read of recent isolations of 11 strains of Tacaribe from 850 bats by Downs and 6 strains of different arboviruses from 300 bats by Williams. In this way, it has been proved in different parts of the world that bats are an important reservoir for several types of arboviruses. As many of these winged mammals are migrant, one can see the danger they represent.

One strain was isolated per average 77 bats processed. Perhaps this figure is underestimated, as bats were pooled in groups of up to 20 animals or slightly more (total 142 pools). But 98 negative pools showed that one must process at least about 100 animals before stating that bats in some locality are free from arboviruses. That conclusion, of course, is relative to Senegal for the years 1962-63.

The Dakar strain 249 is widely scattered throughout Senegal. Perhaps it is in the rest of Africa also because Williams has found the same strain at Entebbe. No isolation is yet available from human material to ascertain whether it is pathogenic. Nevertheless, a human serological survey in Senegal showed HI antibodies effective against only that strain in group B in 3 of 100 inhabitants. Dakar 249 HI antibodies are commonly found to be heterologous in man. In bats, 90 of 165 sera pools (5 to 10 sera per pool) exhibited Dakar 249 HI antibodies which sometimes reached a titer of 1:1280.

The Chikungunya strain is less frequent. HI antibodies in bats were found in 5 of 165 pools, but reached a titer only of 1:10.

During isolation work, it was often noticed that salivary glands were positive, although serum and brown fat were negative. No conclusion has been reached about brain tissue. Many suspensions of salivary glands were toxic for baby mice. Such toxicity decreased by mixing with an equal amount of normal rabbit serum.

Publication: P. Bres and L. Chambon - 1963. Techniques pour l'etude de l'infection naturelle des chauves-souris par les arbovirus. Interet epidemiologique au Senegal. Ann. Inst. Pasteur (a paraitre Juin-Juillet).

REPORT FROM DR. HARRY HOOGSTRAAL
MEDICAL ZOOLOGY DEPARTMENT, NAMRU-3, CAIRO, EGYPT

A large number of Haemaphysalis ticks from eastern Asia are now being studied and all available type material for species from this geographic area is being examined (in conjunction with Dr. Harold Trapido). Excellent progress is being made on a revision of this genus, which will be presented as a 12,000 to 15,000 page monograph in a few years. While a greater number of associations of larvae, nymphs, males, and females of species in this genus are

available than for any other ixodid genus, more material of this sort is solicited from Asia.

New data and material of bird-parasitizing argasids are rapidly becoming available. "Argas persicus" of North America is being studied by Mr. Kaiser (on leave at Emory University). "Argas persicus" reported from tree roosts of wild birds in Pakistan by Abdussalam and Sarwar now proves to be a new species related to A. arboreus; this is being described in collaboration with Mr. Victor McCarthy of the Pakistan Medical Research Center. Dr. Bruce McMillan of the Sydney School of Hygiene and Tropical Medicine has sent us a new Ornithodoros species that parasitizes parrots in Queensland; this is also being described. Dr. B.M. McIntosh of the South African Institute for Medical Research has sent samples of "Argas persicus" from heron rookeries; these are from populations from which Dr. McIntosh has recovered both Quaranfil and Nyamanini virus and are identical with Argas arboreus as described from Egypt. Other argasids are under study from eastern and western Africa, Madagascar, India, Nepal, Iran, and elsewhere. The role of all of these species as vectors of viruses should be investigated.

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

In collaboration with Prof. A. Valle, Director of the Zoologic Museum of Bergamo, researches have been carried on with the aim of isolating arboviruses from arthropods taken from Rattus norvegicus and Rattus rattus captured in the Province of Bergamo. No virus has been isolated (June 30, 1964).

Some research was started in order to study Semliki Forest virus density cultivated in various host cells.

Fractions obtained by density gradient centrifugation (sucrose ans CsCl) were examined for infectivity and experiments were carried out to determine the effects of various host cell types on virus density. The specimens of virus examined by density gradient centrifugation were taken from the brain of suckling mice, HeLa cells, and hamster kidney cell and chicken embryo cell cultures. The experiments are still in progress.

Some experiments to show a sedimentation in the hemagglutinating Ntaya and Ilheus viruses by the sucrose gradient ultracentrifugation of infectious particles have also been carried out.

REPORT FROM DR. HANS MORITSCH
HYGIENE INSTITUT DER UNIVERSITAT
VIENNA, AUSTRIA

Experimental study on the viremia of TBE virus in squirrels:

A study was carried out to get some data on the possible role of hibernating ground squirrels for overwintering of TBE virus. A number of active and hibernating animals which were previously trapped in the field were infected with varying doses of virus.

None of the animals died nor showed signs of disease. However, all developed viremia and subsequently neutralizing antibodies. In active ground squirrels, a high virus dose (10^5 LD_{50}) provoked a short viremia (2-5 days), while small doses (10 LD_{50}) prolonged the duration of viremia till 7 days. In hibernating ground squirrels, both the incubation period and the viremia were considerably longer than in the former, particularly if a small amount of virus was inoculated. However, viremia never persisted longer than 46 days. Therefore, ground squirrels cannot be considered a potential winter reservoir for TBE virus (A. Radda and G. Pretzmann, Zbl. Bakt., I,0, in press).

Investigations in the epidemiology of TBE with calculation of the annual infection rate in an endemic area:

From 1956 through 1963, the rate of tick-borne encephalitis was determined among all patients hospitalized in the hospital of Neunkirchen under the diagnosis of an infectious disease of the CNS. The results, given in Table I, show that TBE regularly accounted for about 50% of all non-bacterial infections of the CNS. In this district with 87,987 inhabitants, all patients with infectious diseases are hospitalized in this hospital. Thus our study gives a valid information on the morbidity in an area where TBE is endemic.

Table 1
Nonbacterial Infections of the CNS in the District of Neunkirchen, 1956-1963

	Tick-borne Encephalitis	Other Diseases of the CNS
1956	44	36
1957	31	22
1958	14	15
1959	12	10
1960	30	34
1961	40	14
1962	16	10
1963	26	22
Total	213	163

Percentage of tick-borne encephalitis: 56.6%
Percentage of other diseases of the CNS: 43.4%

As it has been stated in a previous report, the sera of 1412 normal residents living in the same area were tested in the NT. Fourteen per cent of the sera exhibited neutralizing antibodies against TBE virus.

Subsequently, both data (rate of morbidity and rate of antibodies among normal persons) were compared. On the basis of these findings, it was calculated that only 8.2% of all persons who become infected actually develop disease (E. Groll, J. Krausler, Ch. Kunz, and H. Moritsch, Arch. ges. Virusf., in press).

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

The present report deals with the alimentary infection of hedgehog with tick-borne encephalitis (TE) virus under experimental conditions.

The hedgehog (Erinaceus roumanicus centroeuropaens) in which the probability of an alimentary infection in view of its trophic relations is the most pronounced was used as a model. Two experiments were carried out: one in August at a temperature varying from 19-23°C; the other in November at a

temperature from 15 to 18°C. Two and three hedgehogs were used, respectively.

Viremia was demonstrated in summer from the second through seventh days; in autumn from the second to the tenth day after inoculation. Neutralizing antibodies were found in all animals in titers from 1:8 to 1:64.

The experimental alimentary infection of the hedgehog has, under certain conditions, an analogy in nature. The hedgehog may capture the micromammal-reservoirs of TE virus, e.g. Micromys minutus, Clethrionomys glareolus, Apodemus flavicollis in the course of viremia, when they are slack, less mobile, and less alert. Alimentary infection of the hedgehog thus increases the probability that the hedgehog could become included into the circulation of TE virus in nature.

Man often brings the hedgehog into its home. If such a hedgehog is in its viremic stage because it is infected by different I. ricinus instars, the latter could, after they had fallen off and undergone metamorphosis in the vicinity of the man's housing, become a source of infection for man and some domestic animals (goats).

Reference: Kozuch, O., and Nosek, J. (1964): Alimentary infection of the hedgehog with tick-borne encephalitis (TE) virus. Acta. virologica, 8:284.

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

The Tahyna virus:

Susceptibility of various cell lines to Tahyna virus infections was examined (Dr. L. Sefcovicova). Multiplication of a neuroadapted variant of Tahyna virus accompanied with cytopathic effect was observed in cultures of HeLa cells and in lines derived from monkey heart (CMH), lamb kidney, embryonic bovine liver, rabbit lungs, and rabbit kidney. L cells were less susceptible. In pig kidney cells, no cytopathic effect was noted.

A simple virus neutralization test in rabbit lung (Zp) cells by the simultaneous infection (by setting up the cultures from a cell suspension mixed with viral material) was developed. The results obtained could be compared with the test in primary cultures of chick embryo cells. The titer of virus in simultaneously infected Zp cells was lower by 0.8 to 1.5 log. units than in monolayer of Zp cells and chick embryo cell cultures.

In chick embryo cell cultures under agar, the formation of plaques by the neuroadapted strain of Tahyna virus was influenced by the presence of polysaccharide inhibitors. By adding 600 ug/ml of protamine sulphate (BDH Poole, England), this effect could be eliminated. Washing the agar (Bacto agar Difco Standardized) with saline and distilled water did not remove the inhibitors completely. The optimal plaque formation with Tahyna virus was obtained with washed agar and 300 ug/ml of protamine sulphate.

The direct fluorescent antibody technique was used in studies on the virus antigen production and distribution of Tahyna virus in susceptible chick embryo cells - cultures (CEC) (Dr. Z. Wallnerova in collaboration with Dr. P. Albrecht from the Virological Institute of the Czechoslovak Academy of Science in Bratislava). The conjugate for this method was prepared from the globulin fraction of a rabbit hyperimmune serum which neutralized 3.0 log TCD_{50} of homologous virus in a 1:512 dilution when tested on CEC cultures.

First fluorescent antigen was demonstrated 8 hours after infection (multiplicity of infection was about 10) as minute fluorescent points distributed evenly throughout the cytoplasm. From 10-24 hours after infection they concentrated near the nucleus, coalesced, and finally formed rough, brightly fluorescent granules in the paranuclear area.

Multiplication of Tahyna virus, which was observed only in the cytoplasm of infected cells with typical paranuclear localization of viral antigen, was, for this virus, characteristic.

In a serologic study, hares (Lepus europeaus) and domestic rabbits (Oryctolagus cuniculus domesticus) were investigated for neutralizing antibodies to Tahyna virus (Dr. A. Simkova).

Neutralization tests with sera from 161 hares and 228 rabbits were performed in Zp cells.

The incidence of neutralizing antibodies in hares and rabbits from areas with a mass occurrence of mosquitoes was significantly higher (in hares 32-66%; in rabbits 8-10%) than from areas without mass mosquito incidence (in hares 8%; in rabbits 2%).

To elucidate the overwintering mechanism of Tahyna virus, the role of hibernating hedgehogs as potential long-term reservoir animals of Tahyna virus was investigated. It has been found that hedgehogs (Erinaceus centroeuropaeus roumanicus) are susceptible even to small amounts (8-20 i.c. mouse LD_{50}) of Tahyna virus inoculated subcutaneously. Viremia lasting for 6-10 days was demonstrated in the infected hedgehogs. After a hibernation of 21 to 140 days duration (started at once after the experimental infection) a viremia of the same duration was demonstrated. The virus levels in the blood from individual animals (up to 4.0 log LD_{50}/0.03 ml) can be considered sufficient to insure the infection of mosquitoes.

The role of horses in the circulation of Tahyna virus was followed in further experimental studies (Dr. V. Bardos in collaboration with Dr. J. Jakubik from the Veterinary Research Institute).

Eight foals 3 to 17 days old were inoculated subcutaneously with 2.5, 3.0, resp. 3.5 log. of the "extraneural" strain 236 of Tahyna virus. Viremia lasting from day three to day eight in all foals was measured by i.c. inoculation of daily sera into young white mice. The viremia reached values of 1.0 to 1.5 log. in five foals.

The Calovo virus:

The role of horses in the circulation of Calovo virus was followed in further experimental studies (Dr. V. Bardos, in collaboration with Dr. J. Jakubik from the Veterinary Research Institute).

Four foals 22 to 25 days old were subcutaneously inoculated with 1.2 resp. 2.2 log. of the neuroadapted strain "184" of Calovo virus. Only traces of virus could be detected in the blood of three foals by i.c. inoculation of young mice on the day five, resp. seven, resp. eight.

References:

Sefcovicova, L.: Cultivation of the Tahyna virus in Rabbit Lung Tissue Cells and Its utilization for the virus neutralization test. Csl. Epidem. 13, 153-158, 1964. (in Slovak with English summary).

Wallnerova, Z., Albrecht, P.: Detection of Tahyna virus in Tissue Cultures by the Fluorescent Antibody Technique. Acta virol., 1964, in press.

Simkova, A.: Tahyna Virus in Hedgehogs. Acta virol., 8, 285, 1964.

REPORT FROM PROF. S.R. PATTYN
PRINCE LEOPOLD INSTITUTE FOR TROPICAL MEDICINE
ANTWERP, BELGIUM

Studies on the possibility of installing latent virus infections in experimental animals:

Animals and viruses used were baby chicks, hamsters, rats, rabbits, mice and WEE, Sindbis, Semliki forest, West Nile, and RSSE viruses.

The general plan of the experiences was as follows. Virus was administered by S.C., I.V., or I.P. routes, in amounts which did not kill the animals. Viremia was followed. Weekly during 1 month, or biweekly during 2 months, groups of 2 or 3 animals were killed and tissue cultures made with some organs, always kidneys and spleens, sometimes also heart and lungs. T.C. was made along 3 different techniques: Maitland type, trypsinization and clot cultures attached to the glass. At the same moment, a suspension was made of some fragments of the same organs and this was inoculated into mice or CETC to reveal the eventual presence of virus.

Tissue cultures were observed for one month and at each medium change, an aliquot of the medium was inoculated into susceptible systems to disclose virus appearing in vitro. In no instance was a latent infection found. Virus persisted regularly for a few days in the kidneys, when it was no longer demonstrable in the blood. In these cases, the virus appeared also in the medium of the tissue cultures made from this organ.

In no case did virus appear in the tissue culture medium of organs which had not been shown to contain virus by direct inoculation of the organ suspension.

The mosquito capture program was continued. During winter months mosquitoes were found in stables and cellars. Since the spring, mosquitoes are being captured with the use of a calf as bait. Thirty-five batches of mosquitoes have been inoculated into baby mice.

Serology:

Two hundred sera received from Dr. Bres in Dakar were examined in the plaque neutralization test using paper discs against WEE, Sindbis, Semliki Forest, Middelburg, and chikungunya viruses. The overall results are as follows: WEE 0/190; SF 3/186; chikungunya 166/179; Middelburg 0/149; Sindbis 0/182.

Dr. Bres in Dakar had tested these sera in HI against chikungunya antigen. There is a satisfactory correlation between the two series of results as is shown by the accompanying table.

HI		PNT -	PNT +	PNT ++
Neg	17	7	10	0
20	15	4	10	1
40	11	1	9	1
80	20	0	16	4
160	24	0	17	7
320+	92	1	13	78
Tot.	179	13	75	91

+ = zone of inhibition 3 mm.

++ = zone of inhibition 4 mm. and more.

Cell receptors for arbovirus:

Extracts of tissue culture cells, or organs of experimental animals and mosquitoes were tested in their action on Semliki Forest virus. Most work was done on the reduction of infectivity, but preliminary observations showed that the HA activity can also be suppressed by cell extracts.

In brief, cell extracts from a 10% suspension of organs or a certain amount of T.C. cells or mosquitoes are incubated at 37°C for various time lapses after which residual infectivity is measured by plaque production.

It has been found that cell extracts prepared by a Mickle tissue desintegrator are more active than extracts prepared by 7 cycles of rapid freezing and thawing, and that heating of the cell extracts at 60° C for 60' before contact with virus only partially destroys the "cell receptors".

HA reducing activity of the same organ extracts is more temperature sensitive as it is completely abolished after treating at 60° for 60'.

Fluorescent microscopy:

Appearance of fluorescent antigens was studied in monolayers of CETC cells infected with SF, Si, YF, WN, and RSSE. The indirect staining method was used.

For SF and Si virus antigen was observed in the cytoplasm 6 to 8 hours after infection which corresponds with the maximum virus titer observed in growth curve studies performed earlier.

The effect of low and high bicarbonate contents of the growth medium was also studied, the effects were found to be parallel with those of earlier performed virus titrations.

Fluorescent intra-cytoplasmic antigen was also observed with WN virus, but after a longer incubation time of 3 days.

Intranuclear fluorescence was observed with neurotropic yellow fever virus. No results were obtained yet with RSSE virus.

Efforts to show fluorescent antigens in experimentally infected animals encounter the great difficulty of the danger of possible laboratory infection, when frozen sections are to be used. We therefore tried to work on paraffin sections from fixed tissues. However, no results were obtained yet with this method.

REPORT FROM DR. PAUL M. OSTERRIETH
LABORATOIRE DE MICROBIOLOGIE GENERALE ET MEDICALE
UNIVERSITE DE LIEGE, BELGIUM

From the bacteriolytic culture filtrate of Streptomyces albus G called Actinomycetine (Welsch, 1937), an enzyme complex was extracted. This complex possesses antiviral properties. The loss of infectivity after in vitro treatment of the viruses by the complex was chosen as indicator test of antiviral activity. The sensitive viruses showed at least a 99% drop in titer after incubation for 90 minutes at 37°C with the enzyme complex (final complex concentration: 0.1 mg per ml). The resistant ones showed no drop of titer in the same conditions. Our results are summarized in the table (+ means sensitive virus, - means resistance).

Virus Strain	Sensitivity to the Complex
Poliovirus type 1	-
Coxsackie A type 11	-
Coxsackie B type 5	-
Echo type 7	-
Columbia SK	-
Adenovirus type 1	-
Vaccinia	+
Pseudorabies	+
Semliki Forest	+
Western equine encephalitis	+
West Nile	+
Bunyamwera	+
Tahyna	+

All the sensitive viruses are, or are supposed to be, enveloped, whereas all the resistant ones are described as naked (Lwoff and coll., 1962). The complex inactivates only viruses containing phospho-lipo-proteins, probably from cell membrane origin.

The evidence is that we are dealing with an enzymic activity. The inactivation kinetics has the characteristics of enzymic reactions. The inactivation depends strongly on the ionic strength, pH and ionic composition of the medium. The active substances have a high molecular weight and their activity is destroyed by heat. The reaction is specific as shown by the antiviral spectrum.

After fractionation of the enzyme complex by zone electrophoresis, it was observed that the antiviral activity was restricted to two fractions, each of them containing a protease. One of these fractions, called C, was active on all the viruses which were tested and found to be sensitive to the whole complex (SF, WEE, WN, vaccinia, and pseudorabies). This fraction is therefore responsible for the antiviral spectrum of the complex. The other fraction, called B, destroyed only the infectivity of SF, WN, and pseudorabies. The infectivity of WEE and vaccinia was not impaired. The two arboviruses of Group A behave differently in presence of fraction B. Semliki Forest, but not western equine encephalitis, is inactivated. Further experiments are needed to know if fraction B can be used in diagnostic work to differentiate Group A arboviruses one from another.

Using the method of Richter and Wecker (1963), we observed that sodium deoxycholate liberates from the Semliki Forest virus particles an infective principle which has some of the properties of infectious RNA: sensitivity to RNAase and better plating efficiency when M sodium chloride is used as plating medium.

Experiments on the heat inactivation of Semliki Forest virus suspended in 0.005 M sodium phosphate buffer pH 7.0 and in the same buffer containing 5% W/V sucrose showed that the presence of sucrose (as that of bovine plasma albumin, gelatin or glycerol) decreased the thermal inactivation rate, in this case by a factor 3. In both media, the heat of activation was the same, and very low: $\pm$ 11,500 calories/mole.

Lwoff, A., R.W. Horne, P. Tournier (1962). C.R. Acad. Sc. 254, 4225.

Richter, A., E. Wecker (1963): Virology 20, 263.

Welsch, M. (1937). C.R.Soc. Biol. 126, 244.

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

Change of Address:

As Dr. Gordon Smith has been appointed Director of the Microbiological Research Establishment at Porton, the Arbovirus Research Unit is moving there and the address from 1 August 1964 is:

Microbiological Research Establishment
Porton
Nr. Salisbury, Wilts.

Studies of louping ill in Ayrshire:

a) Small mammals. Trapping this year was in May to contrast with previous studies in April. Comparing the tick infestation of small mammals during the 3 years, the following pattern of infestation emerges: in March, the predominant infestation is of Ixodes trianguliceps; in late March and April, nymphal I. ricinus is the more common and by May, the most common tick is larval I. ricinus. Average infestation rates with I. ricinus in the common species were: Apodemus sylvaticus 68%, Sorex araneus 48%, Microtus agrestis 40%. The average number of ticks per animal was 4-6 for the various species with maxima per animal of 14-30. It will be recalled that louping ill virus has been isolated from A. sylvaticus and S. araneus.

b) Tick population dynamics. A comparison of the 1964 collections with the same period in 1963 seems to bear out our forecast last year that 1964 would be an "adult year", i.e., that there would be proportionately more adults in 1964 than in 1962 and 1963. In view of this, we continue to predict that 1965 will be a "larval year" and 1966 a "nymphal year". A year with a high nymphal population is probably a year with a high risk of louping ill in sheep and lambs. Collections from sheep confirm these findings. They showed that not only were there more ticks on the sheep in 1964, but the nymph/adult ratio in April was 1:4 compared with 1:1 in 1963.

Studies of encephalitis in Sarawak:

Studies so far suggest the following preliminary conclusions:

a) Infections with Japanese encephalitis and dengue viruses are common in all areas so far studied in Sarawak. On the average, about 40% of the population has been infected with one or more of these viruses by the age of 10-15 years, suggesting annual infection rates of the order of 4-5%.

b) More than a third of human illnesses clinically described as encephalitis are due to Japanese encephalitis. The number of cases suggests that the vast majority of infections do not cause encephalitis.

c) The results (together with information for other parts of Asia) suggest that Culex tritaeniorhynchus may be the main mosquito infecting man and attempts continue to isolate the virus from this species. The closely related Tembusu virus has been isolated twice from it and experiments in monkeys suggest that Tembusu is a possible cause of encephalitis in man. If C. tritaeniorhynchus indeed infects man with JE, then higher infection rates would be associated with rice fields and these would probably have a maximum during the period when the rice fields are flooded.

d) Pigs are apparently infected much more frequently than man with JE. This correlates with the preference of both C. tritaeniorhynchus and C. gelidus for pigs over man. The higher preference of C. tritaeniorhynchus for avian blood suggests that birds may also play a part in this infection. The pig may well act as a link host between birds and man, infecting large numbers of mosquitoes in the peridomestic habitat.

e) Dengue infections are commonest in the coastal swamp region where Aedes mosquitoes are commonest. Infections are, however, present everywhere, as is Aedes albopictus. Aedes aegypti is present in Kuching in quite large numbers and probably in all other seaport towns.

f) Bunyamwera group infections are common in pigs, less common in man. However, at least one case of febrile illness in man due to this virus has been detected.

g) Group A arbovirus infections are also commoner in pigs than man but their importance has not yet been evaluated.

A further visit was made in April/May, 1964. Collections included over 73,000 mosquitoes, 535 human sera, 414 pig sera, 333 sera from rodents, and 148 from birds. This material has not yet been processed.

REPORT FROM DR. R. LEWTHWAITE
TROPICAL MEDICINE RESEARCH BOARD
DEPARTMENT OF TECHNICAL COOPERATION
LONDON, ENGLAND

The East African Virus Research Institute, Entebbe, Uganda:

Malignant lymphoma. The Director, Dr. A.J. Haddow, reports the increasing tempo of the investigations of this mutilating tumor, the incidence of which in African children in Uganda was first brought into prominence by Mr. Denis Burkitt, a Surgeon in the Uganda Medical Service, and which has been shown increasingly to occur in other territories in Africa. Dr. Haddow records that in collaboration with a unit from the Imperial Cancer Research Fund (ICRF) working within the Institute, the Uganda Ministry of Health, Makerere University College, a Medical Research Council cytological team and various Mission Hospitals, an intensive investigation of this tumor is now the main project at the Institute. Maps have been made delineating the climatic zone in which the tumor occurs on the African mainland. It now appears that outside this area, which comprises those parts of Africa where the mean annual rainfall is under 20 inches or the mean temperature of the coolest month is below 60°F or 15.6° C, such cases as do occur are usually associated with large bodies of permanent water--rivers, lakes, or swamps.

In a study of the age-incidence of Mr. Burkitt's cases, Dr. Haddow recognizes the following main groups: males, females, cases in which the primary tumor is located in the jaws or orbit, or, in contrast, elsewhere. In the jaw and orbit cases, both males and females show a very sharp peak in the age-grades 4-8 years, the median in both sexes being in the 7th year; in the other group, however, the peak occurs later, the median for males being in the 8th year and that for females in the 9th year. As by this age, the female may be entering what might be called the pre-pubertal years, the question of hormonal influence may arise, particularly with reference to the tumor site, the ovary being the commonest in this group. In the jaw and orbit group, it

has been found that the cumulative age-incidence in the cases studied is reasonably well expressed as a 50 per cent increase per year till age 5-6, and thereafter an involvement of 25 per cent of the remainder per year. Thus virtually all those who will go down with the tumor will do so before age 20 (of 360 cases available for study, only 6 or 1.6 per cent have been over 20 years of age).

So far there is nothing in the age-incidence or geographical distribution to conflict with the view that an arthropod-borne virus (arbovirus) may be involved. The distribution of the tumor corresponds fairly closely with that of Anopheles gambiae and A. funestus. Till a few years ago, this would have been regarded as merely coincidental. Since their incrimination as the vectors in the great O'nyong-nyong epidemic, however, they must be watched much more closely where other arbovirus infections are concerned.

Serological study of patients and relatives continues. The incidence of immunity to Bunyamwera group viruses among patients and their relatives is 39 per cent, as opposed to the 3-4 per cent shown by the control sample and by previous surveys in East Africa. Studies of BUN group viruses, using tissue culture and electron microscopy, have therefore begun in collaboration with the ICRF at Mill Hill, London.

Field work has been concerned mainly with the study of dwellings where cases have occurred and of the environment within a 2-mile radius, in the hope of finding some common factor. Though the number of sites examined is not yet great (16), it is already apparent that many environmental features can be eliminated. So far, indeed, there have been only two common factors--the presence of permanent surface water within half a mile of the hut concerned, and the presence of some form of dense vegetation (forest in Buganda, dense undergrowth in West Nile). In a survey of infected sites in the latter district, one village was found in which three cases had occurred within a 500 yard radius. This is considered a most important finding and Institute plans for the next few months center largely on a detailed investigation of this area. Fortunately, aided by Dr. E.H. Williams, who works in West Nile, another area has been located with similar population density and vegetation, in the same district, where the tumor is unknown. This will form the first control; the second will be a mountainous area in southwest Uganda, where also the tumor is unknown.

The general plan is to collect large series of sera from all three areas, covering the age and sex distribution as fairly as possible (on the basis of the last census), thereafter to screen the samples against all available arboviruses, and finally to adjust the results against a standard population (that of Uganda as a whole, in the last census). This work will take almost the entire output of the mouse colonies (and most of the working time of the laboratory staff) for several months. Even a negative result will be of value, as this would indicate that (a) an arbovirus is not involved, or (b) that the condition, if caused by a virus, is caused by one unknown to this Institute.

In laboratory studies, material from 48 biopsies of cases or suspected cases has been processed; the Institute is using mice and rhesus monkeys, the ICRF is using tissue culture, hamsters, and grey monkeys. The Institute has isolated so far three strains of virus from tumor material (the ICRF picked up two in parallel in tissue culture). All have been Herpesvirus hominis or a very closely related agent. Though this virus is not, on epidemiological grounds, considered likely to have a direct causative relationship to the tumor, these isolations at least show that some viruses do not elude isolation. The first strain isolated was tested against 52 arboviruses (both African and non-African of groups Ab, B, C, BUN and ungrouped) and also against EMC, and Theiler's FA and GD VII mouse viruses, before final identification as herpes. This gives an indication of the increased potential of the reference laboratory facilities of the Institute.

O'nyong-nyong. The major epidemic of O'nyong-nyong fever continues to be an important preoccupation. Over two million cases have occurred since this painful disease, previously unknown, first swept through Uganda. It has now moved 1,500 miles southward and is active around Zomba in Nyasaland. It has also occurred this year in Tanga Province, Tanganyika, which has been visited by Institute staff; four strains of the virus were obtained there. About 2,000 sera have been tested, immunity being found all the way along the coast from Mombasa to Dar-es-Salaam. Outbreaks have also been reported from the Morogoro area of Tanganyika where the disease was prevalent in 1960/61, and from Hoima in Uganda, an area not previously affected.

Chikungunya. This virus infection continues around Entebbe, yielding 19 further isolations from patients. One strain came from a nun at Nabbingo, outside the general area of activity. While most of the virus activity was in July, 1961, there has been a steady trickle of cases since then. There have also been isolations of Chikungunya from mosquitoes taken near Entebbe, two from pools of Mansonia fuscopennata and two from mixed pools of M. africana and M. uniformis. An epidemic among school children from the northern end of Lake Nyasa also seems to have been due to this virus.

Virus Isolations from Man. Further studies on isolates previously discussed have shown that SE 2175, isolated from a sick laboratory worker, is Nairobi Sheep Disease (presumed laboratory infection) and SE 65 (also from man) is a strain of Semunya virus. The agent previously reported as Nakiwogo (from man) has proved to be yet another strain of Semunya. One definite case of Bunyamwera infection (headache and joint pains being the main features) has been recorded and another infection by a member of the BUN group, not yet finally identified. Considerable interest attaches to five cases of Sindbis virus infection in man, these being the first to be studied clinically. The illness was mild in all, but two showed slight jaundice. A laboratory infection with Zika virus in one of our European staff was of interest in that a distinct rash developed.

The clinic. The number of patients now seen per year is some 18,000. Isolations of virus average one to three per month, though they tend to occur in groups.

Outbreaks of disease. Two mysterious outbreaks were investigated. One (a skin disease) was found to be non-viral in origin. The other (Kashasha disease) remains obscure and has been studied by local medical authorities as well as by the Institute.

A more important outbreak, classed as "Clinical influenza", started at Minakulu, Lango District, near the Acholi border on 6th October 1962. A peak in case-incidence was reached about 16 days later when 150 cases were being treated each day. The epidemic did not spread widely, but affected approximately 1,600 of 10,000 people in the area. There were 13 deaths, 6 being among children. As soon as the Institute was notified, blood samples and throat washings

were collected (October 17th) and 21 days later paired convalescent blood samples were taken. Virus isolation was attempted in infant mice and in eggs without success, and HI antibody screening with 9 arbovirus antigens showed no significant change in antibody titers. Dr. Tyrrell, Salisbury, England, offered to screen some paired sera, and has reported that he obtained no evidence that the outbreak was due to Influenza A2, B; Q fever; adenovirus or psittacosis; that it was probably not due to any of the parainfluenza viruses, and that another virus must be sought as a cause of this interesting outbreak.

Reference Laboratory Work: As by the end of last year stocks of most of the viruses, antisera, and antigens required for adequate coverage of the African arboviruses had been built up, the trend of work this year has been different. Only a few additions have been made to the collection or "library", namely Usutu and Ukauwa viruses. With the exception of Lagos bat virus, all the registered arboviruses occurring in Africa south of the Sahara are now available. On the other hand, considerable accent has been laid on the production of standardized antisera in really adequate quantities. The material had become so extensive and varied that a complete revision, re-cataloguing, and rearrangement of existing stocks became essential, and is now well under way.

Incoming sera are tested against nine antigens, giving fairly wide general coverage against the virus infections in our group, viz. Chikungunya, O'nyong-nyong, Sindbis, Semliki Forest, Bunyamwera, West Nile, Zika, H.336 (a strain closely related to Uganda S), and yellow fever (1117 sera from the Ethiopian epidemic).

Field and Laboratory Entomology and Isolations from Mosquitoes. The exceptionally high level of Lake Victoria and almost continuous rain have allowed the development of unusually large and varied mosquito populations. Most of the field work centered on the steel tower, newly sited at Zika (Kisubi). The important observation noted in last year's report that certain species of mosquitoes make daily vertical migrations in forest, thus providing a link between the wild birds and mammals of the forest canopy and man and his domestic animals below, has been amply confirmed. A detailed study of this phenomenon in the sunset period is in progress; 24-hour catches with man as bait also continue. Studies on the mosquitoes attracted

to different types of animals(monkeys, birds, rodents, and lizards) have only partially succeeded, owing to the lack of traps sufficiently adequate as yet for quantitative work. However, Mansonia aurites and Culex quasiguiarti clearly prefer birds, Hodgesia cyptopus prefers rodents, and Aedes africanus and A. apicoargenteus prefer monkeys.

The tower has again proved a focus for swarms of mosquitoes and tabanids (gadflies). The gadfly swarms occur before sunrise, and each species appears at a different time and swarms in a highly characteristic manner. Other studies on swarming behavior were made near ground level outside the forest.

Light trap catches on the tower and in the compound were continued, particularly with reference to season and to the influence of moonlight. A common gadfly (Tabanus thoracinus) is among those which swarm regularly above the tower at daybreak. Females are very rarely seen in or near these swarms, but light-trap work reveals them present in the vicinity, and that in flight activity old and young females behave differently. A new study began, using unbaited and unlighted suction-traps, simply as air samplers, to give an unbiased picture of flight activity. The results (many surprising) in this virtually untouched field should be of great interest.

The all-important subject of the recognition and distinction of old and young mosquitoes has received further study. One method is the examination of the parasitic mites which occur on many species. Not all mites have proved reliable indicators; prior prolonged study of the mites of a particular species is essential. Studies on the age composition of mosquitoes in a seasonal context, involving work on about 32,000 specimens, have shown that the highest incidence of parous mosquitoes (potential virus transmitters) occurs just after the main rains. Dissections of a common species of Culicine, Mansonia fuscopennata, show that most parous females bite not less than 12 hours after oviposition, the majority waiting at least 36 hours.

Aedes africanus, the principal virus vector in the area, lives in water in tree holes in the larval stages, as do several other mosquitoes of interest. Accordingly, a study of some of their enemies has been made, particularly of the voracious predatory larvae of mosquitoes of the

genus Toxorhynchites. Before pupation, a single larva of this genus may devour hundreds of larvae (about 250 on an average), destroying any larvae left alive in the tree hole it inhabits. Similar work was undertaken on the predatory larvae of Eretmapodites spp.

Work continued on the oviposition habits of mosquitoes by using trap bamboo containers on the tower and in different habitats at ground level. It is most interesting that a mosquito which bites most actively in the canopy, such as A. africanus, may prefer low tree holes for oviposition, and further, that the choice of level may be associated with actual height rather than with degrees of shelter, light and shade, and the like. Work on oviposition cycles in the forest showed that in A. africanus the cycle resembled that found in the laboratory. In A. apico-argenteus, however, whose biting cycle is irregular and its oviposition cycle in the laboratory poorly defined, a clear biphasic curve was shown in the field.

Apparatus has been devised by which the contributions of individual specimens of Aedes aegypti to the oviposition cycle can be studied. Each mosquito laid its eggs at the expected time, just before dark, but the individual oviposition was distributed at intervals of two or three days. In other words, if a mosquito had completed ovarian development at just "the right time" it would lay; if not, it would wait until the corresponding time next day. This subject was also studied in constant light, where laying was aperiodic, followed by a change to constant dark, after which laying became cyclic. The mosquitoes which completed ovarian development soon after the change oviposited 24 hours later. Those which had not completed development by then waited for a further 24 hours.

No virus was recovered from the tower during a year's work in Mpanga Forest; but since it was moved to Zika Forest, it has proved a most effective tool for virus recovery, 5 strains of Chikungunya virus being isolated from A. africanus taken on the tower, 5 from people engaged in work there, and 11 strains of Zika virus from A. africanus, and one strain of an unidentified Group B virus (probably new) from Mansonia aurites, a species which prefers birds but bites man fairly freely. These isolations were all made between late May and late October, 1962.

One reason for building the tower was to find whether mosquitoes rose above tropical forest, more particularly in the hour after sunset when strong rising "thermal" currents may distribute them widely. At Mpanga, some species at least were present above the forest canopy after sunset; but the tree hole breeding group, which is of special interest, was poorly represented there. At Zika, a long series of 24-hour catches and a detailed series of sunset catches have shown that many species, including virus vectors such as A. africanus, have this habit of rapid vertical migration at sunset, and dissections showed that among them the percentage of parous specimens was high. These, having had at least one blood meal, include all the potentially infected females.

From mid-November, 1961, until six months later, there were no isolations, though mosquitoes were abundant. In late May, 1962, the first of the Zika strains was isolated from A. africanus and by the time that a third isolation had been made, it was clear that a marked focus of activity had developed at Zika. By this time, however, a virus had been isolated from a Burkitt's lymphoma biopsy, and demanded every available mouse. Seven isolations had been made before it was again possible to inoculate a series of mice with A. africanus by separate levels and by subdivisions of the 24-hour cycle. Thereafter, four more isolations were made, three of them from mosquitoes taken at heights of 80-100 feet (i.e., 10-30 feet above the canopy) in the early night. The most striking were an isolation from a single mosquito taken at 100 feet in the hour 19-20 (second hour after sunset) and one from a group of two taken at 80 feet, also in the hour 19-20, but in another catch. The third high level isolation was from a combined pool taken at 80 and 120 feet. The last isolation came from specimens taken at ground level, but by day. Dissection has shown that the largest accumulation of parous females by day is at ground level. Here at last is the final proof that infected mosquitoes rise into the air above the canopy just after sunset.

During the year yet another isolation of a virus other than O'nyong-nyong was made from Anopheles funestus. This brings to seven the strains of viruses of varying groups (not all yet finally identified) to be isolated from Anopheles spp. Isolations of O'nyong-nyong were many. Close attention must be paid to Anopheles spp. in future.

Malignant Lymphoma. Dr. R.J.C. Harris, Head of the Division of Experimental Biology and Virology of the Imperial Cancer Research Fund (ICRF), Mill Hill, London, has provided the following summary on the progress made by his group in the investigation of the etiology of the malignant lymphoma which occurs predominantly in children in East Africa (vide paragraph 2 of last year's report). The laboratory investigations are supported jointly by the African Medical and Research Foundation, assisted by the Leverhulme Trust, by the ICRF, the Department of Technical Cooperation, and the East African Common Services Organization (EACSO). Equipment was flown out by the Royal Air Force and extensions to the East African Virus Research Institute were built jointly by the ICRF and EACSO.

The investigation includes collaboration with the entomologists at the East African Virus Research Institute, and with the surgeons and pathologists of the Uganda Government and the Medical Faculty of Makerere College in the search for a possible insect-borne viral causal agent. The laboratory unit of the ICRF began its studies in Entebbe in December, 1961.

The following procedures are being adopted:

a) To search for a virus in biopsies of the tumor by direct isolation in in vitro cultures of suitable human or animal tissues. Such an agent might be one of the known arboviruses which, under specific conditions, could transform human or animal cells in vitro. On the other hand, it is also possible that the agent might be a hitherto unknown virus.

b) To test the cancer-inducing properties of any such virus, or transformed cells, by direct inoculation into suitable test animals, such as hamsters or monkeys.

c) To examine sections of fixed human tumor material (and the corresponding cultured cells) by electron microscopy. This material is fixed and embedded in Entebbe and sent to Mill Hill for examination by Dr. R. Dourmashkin in the laboratories of the Division of Experimental Biology and Virology of the Imperial Cancer Research Fund.

So far, some 30 biopsy specimens of the lymphoma have been examined at Entebbe by Dr. P.J. Simons and his team. From three of these, a virus was recovered which was recognized by Dr. R. Dourmashkin as one of the Herpes group. This identification was confirmed serologically in Africa. Herpes virus is not usually regarded as insect transmitted; it is, however, a very common virus in African children. It is more than likely that it is a passenger in the tumor, for a high proportion of children with acute lymphatic leukemia in Europe are known to secrete it. Under certain circumstances, however, Herpes virus can produce cell transformation, and its role in the etiology of lymphoma in African children--possibly in collaboration with an insect-borne agent--is now being evaluated. In addition, the secretor state of normal children in an area in which the lymphoma is not known is being investigated.

Electronmicroscopic investigations of sections of 19 lymphomas by Dr. R. Dourmashkin have not, thus far, given any evidence of virus in the tumor cells. Four of these tumors, however, have shown non-viral, structural, intracytoplasmic bodies. The identity of these is not at present known.

Geograpnical incidence of malignant lymphoma. Mr. Denis Burkitt, Specialist Surgeon, Uganda Government, has provided the following summary of extensive surveys made by him in Africa, aided by grants from the Department of Technical Cooperation and the Medical Research Council.

In 1961, detailed geographical plotting of the incidence in children exhibiting this syndrome in Uganda, together with an extensive survey over nine countries in East, Central, and South Africa, had provided evidence that this condition was altitude dependent. Since the altitude barrier to tumor occurrence was observed to fall progressively as the distance from the equator increased, it was believed that the actual barrier was one of minimum temperature. This temperature barrier has suggested that some arthropod vector is implicated in the transmission of one of the causative factors of this disease. To obtain more data, more surveys were made, as described below.

Ruanda-Urundi. Medical centers in Ruanda-Urundi were visited in April, 1962. This is the most densely populated country in all tropical or sub-tropical Africa, and over

95 per cent of the population live at an altitude exceeding 5000 feet above sea level. The only evidence of the presence of this tumor that could be found in Ruanda-Urundi comprised records of three cases observed near the shores of Lake Tanganyika at an altitude of only 2500 feet. These observations were consistent with the findings of a survey done in Kivu and Ruanda-Urundi, by Gigase, and confirm previous impressions from East Africa that the tumor is altitude dependent at about 5000 feet near the equator.

West Africa. In May and June, Nigeria and Ghana were visited. In West Africa, altitude variations are relatively small compared with those of East Africa. Differences in rainfall are, however, enormous, varying from under 30 to over 400 inches a year.

Nigeria. The tumor is frequently seen and widely distributed throughout the Western and Eastern Regions. Although it occurs sporadically in the North, the incidence appears to be very much lower. It is considered of particular significance that the condition is virtually unknown in Kano in spite of the very high population density in the surrounding country. Following the visit, illustrated leaflets depicting the main features of this tumor syndrome, together with questionnaires seeking information on tumor occurrence, were circulated to over 200 medical centers, Government and Mission, throughout Nigeria. Replies from over 70 hospitals have confirmed earlier impressions that the tumor is seen much more frequently in the wet South than in the dry North. Tumor distribution in Nigeria appears to be related to rainfall.

Ghana. Experience here emphasized impressions gained in Nigeria. There is evidence that tumor frequency is appreciably higher in the wet South than in the dry North, with one exception. Fewer cases are seen at Accra than at Kumasi, less than 200 miles north. It is suggested that this is due to the very low rainfall, less than 30 inches annually, in the coastal area adjacent to Accra.

This relationship between tumor incidence and rainfall observed in West Africa may reflect a dependence on vegetation, and this would further support the theory of possible vector transmission; and vector transmission suggests a viral etiology.

Leopoldville. The Medical School at Louvanium was visited en route to West Africa. The lymphoma syndrome appears to be quite unknown there. Recent correspondence indicates that in spite of vigilance, it has not yet been observed. An explanation must be sought for the apparent absence of the tumor in an area which would appear to be climatically favorable. It is possible that the highly efficient efforts at malarial eradication carried out by the Belgians may be related.

Therapy. Chemotherapy trials have been very encouraging. Not only has complete clinical resolution of large tumors been observed frequently, but at least two children are symptom-free after three years, and another after nearly two years. These have been traced with some difficulty, suggesting that many others who were discharged from hospital symptom-free may still be well. Methotrexate and Endoxan have been the drugs used.

The Trinidad Regional Virus Laboratory:

The Director of the Laboratory, Dr. Leslie Spence, records an important change in its administration, namely, that during the year it became an integral part of the Department of Microbiology of the Faculty of Medicine of the University of the West Indies. The Director received the additional designation of Senior Lecturer in Microbiology at the University, and a junior member of his staff the designation of Lecturer. The source of funds remains as formerly, being shared in approximately equal fractions by The Rockefeller Foundation and by the West Indian and United Kingdom Governments. The Rockefeller Foundation generously made an additional grant for the construction of an insectary. The Government of Trinidad and Tobago gave a grant of 4,000 pounds for the construction of a virus diagnostic laboratory within the main laboratory, and will provide the staff and recurrent expenditure for this additional Trinidad facility.

Scientific activities in Trinidad were concentrated on studies in Bush Bush Forest, Nariva Swamp. In particular, a concerted effort was made to demonstrate continued virus transmission during the dry season. This was partly successful. Frequent isolations were made from mosquitoes, but the source of the infective blood meals of these mosquitoes was not established. However, presumably

rodents served as sources of infection during both the dry and the wet season of the year. During the year, 136 arboviruses were isolated from Bush Bush material, 18 from sentinel mice, 18 from rodents, and 100 from mosquitoes. Among these were 39 strains of VEE virus, 20 strains of Caraparu virus, and 59 strains belonging to the Guama group. St. Louis encephalitis (SLE) virus was encountered once and Ilheus virus 7 times. Also, an agent new to Trinidad was isolated from Ornithodoros ticks collected on Soldado Rock, the first virus to be isolated from ticks in Trinidad.

Other mosquito studies included a series of 25-hour catches at the three three stations both at ground level and in the canopy during the dry season as well as late in the year. Collections of critical mosquito species through the use of double-baited "type 10" traps were increased considerably during the dry season, by adding more traps, locating them where mosquito densities were highest, and increasing the trapping periods. Considerable attention was devoted to investigating mosquito larval biology and relating it to the prevalence of adult populations.

Several projects begun in 1961 were continued. These included studies of mosquito activity as revealed by flap-traps, exposure of various kinds of bait animals in double baited cage traps, and captures of canopy mosquitoes attracted to white mice. Studies also began on new types of traps and on the possible repelling action of traps. A study of longevity and movement of small mammals in the forest began in May. Trapped animals were marked and released at the point of capture. Retrapping has already resulted in significant information.

Two expeditions to neighboring countries were made in response to calls for assistance. Outbreaks of eastern equine encephalitis (EEE) in horses in British Guiana and in Jamaica were investigated by senior staff members accompanied by their technicians. Human cases, some fatal, occurred in Jamaica. Studies on more than 400 bird sera from British Guiana showed low immunity rates to EEE virus. No EEE immunes were found among a small collection of birds from Jamaica.

The laboratory participated in diagnosis in an epidemic of poliomyelitis in British Guiana. Nineteen strains of

Polio type I virus and two Coxsackie virus strains were isolated from specimens submitted from 13 clinical cases and 4 contacts.

Investigations on non-arboviruses continued during 1962. Serological evidence was obtained of activity of Influenza B viruses in the Trinidadian population during 1962, and of past activity of other respiratory viruses, adenoviruses, parainfluenza viruses, and respiratory syncytial virus in Trinidadian children. Viruses were isolated from clinical cases of mumps, rabies, adenovirus type 2, and parainfluenza type 2.

During the year studies included those on Venezuelan equine encephalitis, Caraparu and Guama group viruses on rodent sera collected from 1955 onwards; on equine and human sera collected from the Courantyne coast in British Guiana, for evidence of infection with arboviruses; and on serum hemagglutinins of viruses of the Guama group.

Neutralization tests were made with Cocal, EEE, western equine encephalitis, and VEE viruses in suspended cell cultures prepared from chick embryos. The behavior of Guama virus in KB cell cultures and Cocal virus in KB and chick embryo cell cultures was also studied.

A series of laboratory studies with viruses common to the Bush Bush area, using wild rodents from the laboratory colonies, have yielded important basic facts about their behavior in infected animals and the development and persistence of induced antibodies. Studies with Cocal virus, an agent related to the economically important virus of vesicular stomatitis in livestock, were intensive and have been especially productive. As the epizootiology of each of the agents presently under study is not completely understood, it is of great importance to coordinate field and laboratory studies in such a way that the answers obtained complement each other. During 1962, the combination of these approaches has enormously increased our understanding of underlying mechanisms of virus survival.

Trachoma and inclusion Blennorrhoea:

Field studies in the Gambia. Trachoma vaccine. The Medical Research Council's Trachoma Unit completed its preparations for a full scale prophylactic trial. Because of the high

prevalence of trachoma, it was difficult to collect in a reasonably circumscribed area sufficient children free from the disease; nevertheless, such a group has now been assembled in two villages on the North Bank of the river. It is hoped to start vaccinations in May 1963.

Genital tract and neonatal infections. The Unit continued its observations on babies acquiring trachoma/inclusion blennorrhoea (TRIC virus) infections shortly after birth, and on their mothers.

Role of Haemophilus in trachoma. At the Lister Institute, Miss Georgina Sampson examined 244 strains of Haemophilus bacilli isolated from the eyes of Gambians. It was established that about 25 per cent of subjects carry more than one type of Haemophilus; and that whereas H. influenzae and other strains fermenting indole are equally prevalent in trachomatous and non-trachomatous subjects, H. aegyptius and other indole-negative strains are found significantly more often in people with trachoma.

Researches at the Lister Institute. In collaboration with two British pharmaceutical firms, workers from the Trachoma Research Unit and the Lister Institute continued to develop methods for the preparation and assay of trachoma vaccine. Dr. L.H. Collier and Dr. W. Blyth found that baboons successfully immunized against LB4 virus during 1960 still had pronounced resistance to infection 15 months later. In an attempt to find a strain of trachoma suitable for use as challenge in such experiments, one Saudi Arabian and eight Gambian viruses were tested for pathogenicity in the baboon conjunctiva, but none induced such severe infections as the inclusion blennorrhoea agents tested previously. At the Institute's Elstree Laboratories, Mr. G. Turner, Dr. Collier, and Dr. Blyth prepared a live trachoma vaccine for use in the forthcoming Gambian field trial.

Drs. P. Reeve and Janice Taverne devised a new method for counting virus particles by dark field microscopy, which is proving useful for quantitative studies of viral growth and purification; it was also used to show that the amount of complement-fixing antigen in a given suspension is directly proportional to the number of elementary bodies, about 10^7 being required to fix 1 unit of complement in the presence of excess antibody. They also found that some TRIC agents give rise to variants that kill chick

embryos comparatively quickly, and that they can be propagated readily in cell cultures and in mouse brain. Miss Elizabeth Fraser began a study of the mechanism by which TRIC agents enter the host cell, and Miss Doris Graham continued to study their serological relationships in terms of the neutralization test in HeLa cells; she showed that antisera to both trachoma strain T'ang and inclusion blennorrhoea strain LBl neutralize lymphogranuloma venereum virus; by contrast, T'ang antiserum fails to neutralize an American and a Saudi Arabian strain of trachoma.

The Arthropod-borne Virus Research Unit, London School of Hygiene and Tropical Medicine:

Encephalitis in Sarawak: Dr. Gordon Smith, with Dr. H.E. Webb of St. Thomas's Hospital, and Mr. W.W. Macdonald of the Liverpool School of Tropical Medicine, spent December, 1962, and January, 1963, in Sarawak. The records of the General Hospital, Kuching, for 1959, 1960, and 1961, revealed 65 cases of encephalitis, mainly in children. The cases seen clinically resembled those elsewhere caused by arthropod-borne and enteroviruses. A temporary laboratory was established near Kuching: mice were air-freighted from Kuala Lumpur, and dry ice from Singapore. Much of the initial mouse inoculation for virus isolation was done in Sarawak. Specimens and sera were later flown to London for further study. Satisfactory pairs of acute and convalescent sera were obtained from 14 encephalitis patients; 7 of these showed diagnostic increases in hemagglutinin-inhibiting (HI) and complement-fixing (CF) antibody to a group B arbovirus related to Japanese encephalitis and Tembusu (MM 1775) viruses; another 4 were possibly also group B infections. Virus isolations were attempted from 36 acute-phase blood samples from febrile patients; a virus, probably dengue, was isolated from 1. Satisfactory paired sera were collected from 27 patients with febrile illnesses; 2 (including the probable dengue patient) and possibly 4 others showed antibody responses typical of group B infections.

From a variety of places, 862 sera were collected, mainly from younger age groups, for serological surveys; 50 pig sera were also collected. Further study of the Sarawak sera collected in 1961 has shown that group A arbovirus infections are much less common than group B infections.

The high prevalence of HI antibody to certain group A viruses previously reported was apparently due to the use of redistilled acetone for serum extraction.

For entomological study, 20,574 mosquitoes of about 90 species were hand caught in tubes coming to bite man at 4 main sites and in a number of minor collections; 2,789 were taken in a pig-baited Magoon trap at a piggery. In all, 18,911 mosquitoes were ground up in 170 pools of about 100 and inoculated into suckling mice. So far, there are 3 definite isolations (and re-isolations) of virus; MS 50 from Aedes (Cancraedes) curtipes; and MS 117 and MS 128 from Culex tritaeniorhynchus. All were from mosquitoes taken at Kampong Pangkalan Kuap; A. curtipes was the commonest day-biting mosquito in the village itself. The C. tritaeniorhynchus were taken at the rice fields about a mile away through secondary forest. Some mosquito species were very common in one area but absent or rare in the others; for example, A. curtipes in Kg. Pangkalan Kuap and Aedes (Skusea) amesii/fumidus in Kg. China. Some, on the other hand, were present to a greater or lesser extent almost everywhere; foremost among these were the night-biting Culex tritaeniorhynchus and Mansonia annulifera, and the day-biting Aedes (Stegomyia) albopictus.

Cross challenge experiments have shown that MS 117 and MS 128 are apparently identical but differ from Japanese encephalitis virus. Further studies are in progress to identify the 4 viruses isolated and to determine whether the encephalitis cases were due to MS 117/128.

Louping ill. Studies of sheep in collaboration with Mr. A.L. Wilson of the Veterinary Investigation Department for the West of Scotland and Mr. K.J. O'Reilly of the Wellcome Research Laboratories have continued. The prevalence of louping ill in sheep was low in 1962. The investigation of ticks and small vertebrates in the same farms with Dr. M.G.R. Varma and financial assistance from the Agricultural Research Council also continued; 130 rodents and insectivores were trapped in March/April, 1963, and are being studied for evidence of infection. Tick collections are being made on drags and on sheep throughout the year so as to relate tick population and activity to vegetation patterns and infection rates. As 1959 was a vole-year and 1960 an epizootic year in lambs, and because the disease occurs in such epizootics at intervals of years, it is

suggested that years of high prevalence are related to the population of small vertebrates in the preceding year. These studies will continue until the next epizootic year so that a full picture of the epidemiology can be built up.

Mr. Chan Yow Cheong, Wellcome Fellow in Tropical Virology, is studying the antigens of louping ill virus by calcium phosphate chromatography and density gradient centrifugation; H.I., C.F. and precipitating antigens are being measured and compared. Different strains of louping ill virus give different and characteristic fractionation patterns by these methods. Miss Fatima Begum is studying the growth, heat stability, antigens, and chromatography of plaque-purified lines of louping ill and Langat strains with different passage histories.

Langat virus. Serological studies have now been completed on the patients with malignant disease who were later infected with Langat and Kyasanur Forest disease viruses for possible oncolytic effects, as reported last year. In the 18 patients successfully infected subcutaneously with Langat virus, viremia was detected 2-32 days after infection but was commonest from the 3rd to the 14th day. Viremia was higher titered and more prolonged in leukemia patients than others. HI and neutralizing antibody first appeared 16-19 days after infection and all patients had neutralizing antibody after 30-36 days and HI antibody after 38-43 days. CF antibody first appeared 20-24 days after infection and was present in all patients after 38-43 days.

Training. During the year several virologists worked in the unit for periods of from 2 weeks to 3 months: Dr. P. Ardoin from Paris, Dr. S.D. Paul from Poona, Dr. M. Pavlatos from Athens, Dr. D.I.H. Simpson (a Colonial Research Student), Dr. L.S. Smith from Cape Town, and Dr. Z.D. Wroblewska-Mularczykowa from Warsaw. Two technicians in training also spent about 6 months in the unit: Mr. R.S. Koshuma from Tanganyika and Mr. Chan Chee-Fai from Hong Kong.

REPORT FROM THE WISCONSIN STATE LABORATORY OF HYGIENE AND DEPARTMENT OF VETERINARY SCIENCE AND ENTOMOLOGY UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

Serologic evidence that Wisconsin residents have been infected with California group virus has been reported by Dr. Wayne Thompson of the Zoonoses Research Unit of the State Laboratory of Hygiene, University of Wisconsin.

One-third (55/155) of sera collected from Conservation Department workers during 1962 had sera which neutralized 100 $TCID_{50}$ or more of California group virus (Montana snowshoe hare isolate) in metabolic inhibition screening tests conducted in cooperation with the Veterinary Science Department. Mouse neutralization tests with some of these sera also showed that these sera neutralized California encephalitis virus (BFS 283) and the related A. trivittatus virus. In studies with additional sera from other occupational groups with relatively great exposure to insects, 167 of 654 sera tested neutralized the California group virus. None of these sera neutralized eastern encephalitis virus, 2 neutralized western, 11 St. Louis, and 17 encephalomyocarditis virus. No evidence was found of recent associated illnesses in these adults. The largest percentage of positives was found in older age groups, in those with the greatest length of service, and in those living in the rural western and northern portions of the state.

Indication of recent virus activity is provided by results of tests with sera of 260 high school age youth attending forestry camps during the summer of 1963. Blood samples were collected from these boys near the beginning and end of these camp periods. Neutralizing antibodies to California group viruses were found in 34 of the 260 sera collected from the boys at the end of the camp periods. Antibodies were not detected in sera collected from four of these boys soon after they arrived at the camps, but were positive in mouse neutralization and HI tests when they left the camps. Three of these boys had illnesses while they were in camp which may or may not have been related to California virus infections.

Relationships between clinical illnesses and the appearance of antibodies indicating recent infections with California group viruses are being studied by an HI test survey of

paired sera received by the State Laboratory of Hygiene for diagnostic purposes from cases of CNS disease. Results of these studies are listed in Table 1. These results are suggestive of a relationship between some of these illnesses and recent infections with California encephalitis group viruses, especially in the first and last listed cases. Similar studies of paired sera received during the previous year, 1960, show similar results involving eight cases of CNS disease in children hospitalized mostly in the west-central part of Wisconsin.

An isolate has recently been obtained from brain tissue of a four-year-old girl who died with encephalitis in a LaCrosse hospital during September of 1960. Although comparative studies are not yet complete, preliminary data indicate that the isolate is serologically related to California group viruses. The isolate is neutralized by anti-California (BFS 283) rabbit antiserum (3.4 logs.) and antiserum to the isolate prepared in guinea pigs neutralizes 2.8 logs of California (BFS 283) virus. Sucrose-acetone antigen prepared from the isolate fixes complement with a 1:32 dilution of antiserum of the Montana snowshoe hare isolate of California encephalitis virus prepared in guinea pigs. Reisolation of the virus has been made in another laboratory, a Veterinary Science Department laboratory, with the assistance of Dr. Ralph Anslow. Further tests of this isolate and of its relationship to convalescent sera of other children ill with a similar type of CNS illness at the same time in the area are in progress.

Table 1: Relationships between California encephalitis virus antibody levels and clinical data in eight Wisconsin children with severe illness.*

Patient age, sex, residence county.	Illness reported by physician	Date of onset	Dates of serum collections	Test results with Calif. enceph. virus antigens		
				HI	CF	Logs LD_{50} of virus neutralized in mice
D.P. 9 yr. M. Vernon	Fever, headache, diagnosed as possible encephalitis or meningitis	7-29-61	8-2-61 8-23-61	<10 40	<4 <4	1.2 4.5
R.M. 4-1/2 yr.M. Walworth	Fever, convulsions, vomiting, stiff neck, diagnosed as aseptic meningitis	8-20-61	8-22-61 9-6-61	(none available for test) 20	<4	3.6
T.D. 10 yr.M. Iowa	Fever, semicomatose condition, moderately severe encephalitis	9-20-61	9-25-61 10-14-61	(none available for test) 40	1:8	>3.3
L.H. 5 yr.M. Monroe	Fever, vomiting, headache for 4 days	9-28-61	10-2-61 10-15-61	<10 80	<4 <4	2.5 3.2
L.D. 9 yr.F. LaCrosse	Headache, convulsions, high fever, vomiting	7-6-62	7-11-62 8-7-62	20 40	<4 1:8	3.2 3.9
K.O. 5 yr.F. Washburn	Recurrent convulsions for 10-12 hours before admission	8-10-62	8-15-62 8-25-62	<10 20	<4 <4	2.4 4.2
T.H. 5 yr.M. Eau Claire	Diagnosed as meningoencephalitis	8-8-62	8-18-62 8-27-62	40 80	<4 <4	3.2 4.3
D.J. 11 yr.M. Vernon	Pleocytosis, stiff neck, diagnosed as viral encephalitis	7-6-63	7-8-63 7-31-63	<10 40	ND ND	1.4 3.4

*Selected by an HI test survey of sera submitted to the State Laboratory of Hygiene for diagnostic tests for CNS syndrome from 68 cases during 1961, 96 during 1962, and from 67 cases during 1963.

REPORT FROM O. MORGANTE, J.A. SHEMANCHUK, AND J.H. BROWN
PROVINCIAL LABORATORY OF PUBLIC HEALTH
UNIVERSITY OF ALBERTA, EDMONTON, CANADA

Serological surveys for infection with WEE, EEE, and SLE viruses were conducted in chickens.

Sentinel flocks of approximately thirty birds in each flock have been placed in different locations of the province in an attempt to define foci of infection. Seven flocks have been placed in the southeast, east, northeast, northwest, and west regions, and four flocks in the south near Lethbridge in a vast, irrigated rural area.

The chicks were hatched in April and bled in May before exposure in the field. They will be bled at monthly intervals until the end of October. So far, 302 sera, collected in May, have been acetone extracted and tested for the presence of HI antibodies to WEE, EEE, and SLE. No serum was positive.

Collection of mosquitoes was made from many parts of Alberta by the staff of the Entomology Section Research Station, Canada Department of Agriculture, which is located in Lethbridge.

Fifty pools of approximately fifty mosquitoes each have been collected in the northern regions of the Province. The pools have been processed and inoculated intracerebrally in infant mice. No virus has been isolated as yet.

REPORT FROM A.N. BURTON, J.R. MCLINTOCK, AND J.G. REMPEL
DEPARTMENTS OF VETERINARY SCIENCE AND BIOLOGY
UNIVERSITY OF SASKATCHEWAN, SASKATOON, AND
THE ENTOMOLOGY RESEARCH INSTITUTE, RESEARCH BRANCH
CANADA AGRICULTURE, OTTAWA, CANADA

Arbovirus Studies in Saskatchewan, 1963:

Our observations indicated that there was considerable WEE virus activity in Saskatchewan in 1963, particularly during the period from the last week of July to the third week of August.

The incidence of WEE in the human and the horse populations was reported in issue number nine of the Arbovirus Information Exchange. The unusual abundance of mosquitoes during the season provided large numbers of specimens for virus examination. Systematic collections were commenced June 1 and were carried on to the 30th of September. During this time, 46,327 female mosquitoes were identified and distributed into 1843 pools as outlined in Table I.

Although Culiseta inornata was more than four times as abundant as Culex tarsalis, only four WEE isolates were obtained from C. inornata as compared to 13 for C. tarsalis. One pool of Aedes dorsalis also yielded a strain of WEE virus. More detailed results are outlined in Table II.

A total of 480 blood samples were collected from wild birds for virus examination. In 1963, as in 1962, a number of WEE isolations (eleven) were made from the blood of English sparrows (Passer domesticus). One WEE isolate was obtained from the blood of a nestling Swainson hawk (Buteo swainsoni) collected July 4.

TABLE I

MOSQUITOES EXAMINED FOR WEE VIRUS, SASKATCHEWAN, 1963

SPECIES	SURVEY TRAPS		MISC. CATCHES		TOTALS		POOLS
	SPECIMENS	ISOLATIONS	SPECIMENS	ISOLATIONS	SPECIMENS	ISOLATIONS	
Culiseta inornata	16184	3	3800	1	19984	4	673
Aedes dorsalis	8228	1	2345	0	10573	1	351
Culex tarsalis	1863	9	2255	4	4118	13	189
Aedes campestris	3236		851		4087		177
Aedes spp.	12		3358		3370		105
Aedes spencerii	198		1561		1759		78
Aedes vexans	309		755		1064		75
Aedes flavescens	298		336		634		69
Anopheles carlei	61		205		266		41
Aedes nigromaculis	155		91		246		41
Aedes punctor	12		64		76		8
Aedes cataphylla	0		43		43		2
Aedes stimulans	27		12		39		13
Aedes riparius	0		26		26		3
Aedes fitchii	8		16		24		10
Aedes exerucians	1		11		12		4
Aedes increpitus	2		1		3		2
Mansonia perturbans	0		2		2		1
Culiseta sp.	1		0		1		1
Totals	30595	13	15732	5	46327	18	1843

1963 - WEE ISOLATIONS - MOSQUITO

NO.	CODE NO.	LOCATION	MOSQUITO SPECIES	NUMBER IN POOL	DATE COLLECTED	DATE FROZEN	DATE OF EXAMINATION
1	M1342/63	Brock	C. tarsalis	40	6:VIII:63		9:VIII:63
2	M1561/63	Milestone	C. tarsalis	30	8:VIII:63	14:VIII:63	22:VIII:63
3	M1382/63	Kindersley	C. tarsalis	40	6:VIII:63	9:VIII:63	15:I:64
4	M1540/63	Outlook	C. incornata	30	10:VIII:63	14:VIII:63	29:I:64
5	M1407/63	Outlook	C. tarsalis	26	8:VIII:63	12:VIII:63	14:II:64
6	M1313/63	Malfort	C. tarsalis	35	3-6:VIII:63	8:VIII:63	29:I:64
7	M1642/63	Outlook	C. tarsalis	42	11:VIII:63	22:VIII:63	11:II:64
8	M1406/63	Outlook	C. tarsalis	24	8:VIII:63	17:VIII:63	19:II:64
9	M1217/63	Forestry Farm	C. tarsalis	42	31:VII:63-3:VIII:63	5:VIII:63	2:III:64
10	M1306/63	Forestry Farm	A. dorsalis	11	5-6:VIII:63	8:VIII:63	27:II:64
11	M1361/63	Brock	C. tarsalis	40	6:VIII:63	9:VIII:63	25:II:64
12	M1345/63	Brock	C. incornata	18	6:VIII:63	9:VIII:63	25:II:64
13	M1288/63	Craik	C. tarsalis	49	4-6:VIII:63	8:VIII:63	15:II:64
14	M1162/63	Outlook	C. incornata	40	27:VII:63	1:VIII:63	10:III:64
15	M773/63	Outlook	C. incornata	30	27:VII:63	28:VII:63	23:III:64
16	M1663/63	Forestry Farm	C. tarsalis	7	15-21:VIII:63	22:VIII:63	28:V:64
17	M1599/63	Craik	C. tarsalis	17	8-12:VIII:63	14:VIII:63	19:V:64
18	M105/63	Outlook	C. tarsalis	4	22:VI:63	24:VI:63	9:VI:64

REPORT FROM J. DITCHFIELD AND L. KARSTAD
DIVISION OF ZOONOSES AND DISEASES OF WILDLIFE
ONTARIO VETERINARY COLLEGE, GUELPH, CANADA

The Virus of Epizootic Hemorrhagic Disease of Deer:

In the late summer of 1962, there was a heavy die-off in white-tailed deer in the southeastern part of the province of Alberta. Post-mortem examination revealed the cause of death to be due to epizootic hemorrhagic disease of deer. A virus has been isolated from specimens received from this outbreak which shows serological identity with the New Jersey strain of EHDD virus. Studies on the characterization of this virus have shown it to be a ribonucleic acid virus of simple cubic symmetry, with a particle size of 25-35 mu. The Alberta virus is chloroform resistant, acid sensitive, and its infectivity is not stabilized by M $MgCl_2$ when heated at 50°C for one hour. The virus shares complement-fixing antigens with the encephalomyocarditis subgroup of the Picornavirus group. It is apparent that the Alberta strain of EHDD virus is not a member of the Arbovirus group, and that a different pattern of epizootiology should be elucidated for this disease of deer.

REPORT FROM DR. DONALD M. MCLEAN
THE HOSPITAL FOR SICK CHILDREN, TORONTO, CANADA

Between May 1 and June 7, 1964, sera from 105 animals collected in forested areas within 40 miles of North Bay, Ontario, were examined for evidence of antibodies to Powassan virus by hemagglutination inhibition (HI) and neutralization (NT) tests. HI antibodies were detected in 37 of 71 groundhogs (Marmota monax), 1 of 8 porcupines (Erethizan dorsatum) and 2 of 5 chipmunks (Tamias striatus), but not in 16 red squirrels (Tamiasciurus hudsonicus) or 5 snowshoe hares (Lepus americanus). Groundhogs with antibody were collected from the following townships: Nipissing (1 of 2 animals), South Himsworth (1 of 3), East Ferris (13 of 23), Bonfield (13 of 25), Chisholm (9 of 12). Antibody-containing animals have been taken from all these townships during previous years. The groundhogs have been usually plentiful during 1964. Most groundhogs were adults, but of 6 juveniles collected, 3 circulated antibody. Of 78 sera examined so far by NT,

including 71 which were examined by HI, 21 showed antibody by both techniques, 2 of which were not tested by HI neutralized Powassan virus, and 2 which had no NT antibody inhibited Powassan hemagglutinin.

Ixodes cookei ticks were removed from 26 groundhogs and 3 porcupines, and I. marxi ticks were removed from one squirrel and one chipmunk. Ticks from two groundhogs yielded Powassan virus following intracerebral inoculation into suckling mice. Blood clots from 27 animals have not yielded virus by this technique as yet.

A program of serial bleeding of tagged fauna in endemic foci in attempt to isolate virus from natural reservoirs has now commenced.

REPORT FROM DR. ROBERT J. TONN, TAUNTON FIELD STATION MASSACHUSETTS HEALTH RESEARCH INSTITUTE, INC. TAUNTON, MASSACHUSETTS

Field studies on the ecology of arthropod-borne viruses in Massachusetts have been transferred from the Communicable Disease Center to the Massachusetts Health Research Institute, Inc. The studies at the Taunton Field Station will continue as before, except virus isolation and antibody testing will continue to be done by the Virus Section, Diagnostic Laboratories, of the Massachusetts Department of Public Health.

A total of 208 blood specimens were obtained from 41 species of wild birds during the 1964 spring migration period (April-June). Permanent resident birds were included in the survey total. The netting area continued to be the dike which traverses Pine Swamp. Sixteen mist nets were used during seven collecting periods. Of these blood specimens, 183 have been tested for virus and no virus has been isolated using chicken embryo tissue culture systems.

The results of the 1963 chickadee study have been received. Using the plaque reducing antibody technique, no conclusive antibody activity was reported for either EE or WE.

Six spotted turtles survived the 1963-64 virus overwintering study. As yet, the blood specimens taken from these turtles have not been tested for the presence of virus.

As in 1963, the attempt to feed Aedes abserratus on turtles was not successful.

The arthropod surveillance studies, sentinel chicken flock studies, and summer autumn wild bird studies are being continued.

REPORT FROM JOAN B. DANIELS, VIROLOGY SECTION MASSACHUSETTS PUBLIC HEALTH LABORATORIES BOSTON, MASSACHUSETTS

As reported elsewhere in this issue, the Taunton Field Station has now become the Diagnostic Laboratory Taunton Field Station, as the Massachusetts encephalitis program becomes unified in the State Department of Public Health. Dr. Robert J. Tonn succeeds Dr. Richard O. Hayes as Entomologist in charge of the Field Station.

Reptiles:

Natural antibody for EE has now been found in a total of three spotted turtles in the relatively small number examined during the program. Bloods from twenty spotted turtles have been recently submitted to the laboratory and more will be collected.

Reptiles may well be an important host or even reservoir (see overwintering studies, Hayes et al., American Journal of Tropical Medicine and Hygiene, in press).

Sentinel flocks:

Sentinel flocks of 35 six-week-old chickens were established in May 1963 at Pine Swamp Study Sites I-IV for comparative ecology studies and the site VI flock for experimental control studies. Six chickens were retained in a screened aviary as controls, their bloods submitted to the laboratory with each flock bleeding.

October bleedings were tested first, then the serial bloods for each positive bird were back-tested to determine the time of conversion. The first conversion was in a single chicken at site III before August 8 (first positive on that date). Site III also had the highest transmission rate; 41% (19% site I, 4% site II, 0% site IV, 19% site VI).

The relatively high transmission rate obtained for WE virus at site III during 1962 and 1963 differs from data obtained in previous years and may be indicative of the involvement of enzootic vector species other than Culiseta melanura. There was no EE antibody conversion in sentinel chickens.

Sentinel flocks were bled thrice weekly in an attempt to isolate virus during August 1962. No viruses were isolated. Since WE antibody conversions had already occurred in early August 1962, the chance of finding viruses in the blood in July seemed more promising. Accordingly, in 1963, 843 bloods were collected in July and August from the flock at site I, but no virus was isolated.

Mouse embryo tissue cultures:

Since the infant mouse is sensitive to most, if not all, arboviruses, it was hypothesized that mouse embryo tissue cultures (METC) might also have a wider viral spectrum than chick embryo tissue cultures (CETC). Pools of wild caught mosquitoes containing WE virus were therefore tested for virus, in parallel, in METC, and CETC. Eleven out of 13 were reisolated in CETC showing rapid and complete destruction of the cultures. Only one mosquito pool produced any cytopathogenic effect (cpe) in METC. Virus was recovered, however, from 7 of the 12 healthy looking cultures by sub-inoculation into CETC. The lower sensitivity to WE virus and the absence of the cpe indicator of viral growth do not suggest that METC will be the universal cell system for arbovirus isolation.

REPORT FROM ELINOR WHITNEY
DIVISION OF LABORATORIES AND RESEARCH
NEW YORK STATE DEPARTMENT OF HEALTH, ALBANY, NEW YORK

Survey of sera from residents of two counties:

The arbovirus survey in New York State was extended in 1963 to include 568 sera, 226 from residents of Schuyler County (a rural area) and 342 from residents of Suffolk County (a suburban area). The sera had originally been submitted to our Division for other tests. Two serologic methods, hemagglutination-inhibition (HI) and suckling mouse neutralization tests, were employed. The technics have been described previously (Whitney, E., Am. J. Trop. Med.

and Hyg., 1963, 12:417-424). The screening viruses were the same for Group A, Eastern and Western encephalomyelitis (EE and WE), and Group B, St. Louis encephalitis (SLE) and Powassan (POW). Cache Valley virus (CV) and Flanders (related to Hart Park) were also included. The CV strain (V62-7364) used for neutralization work was originally isolated by Holden and Hess, and for HAI work, was the Belem strain AR7272. The intracerebral route of inoculation was used for the CV and Flanders neutralization work, the volume inoculated was 0.03 ml., and the mice were one day old. The hemagglutination antigen for CV was prepared by the sucrose-acetone method of Clarke and Casals. No HAI tests have been done with Flanders to date because antigens so far have failed to show hemagglutination.

Results: Thirteen (5.8%) sera from Schuyler County residents had detectable neutralizing antibodies for CV. No sera had CV HAI titers. Group A activity was slight, 2 sera showing some reactivity. One serum neutralized EE virus and the other WE virus. Three additional sera had reproducible HAI titers of 10-20 with WE. Group B activity was lacking. One serum, however, neutralized Flanders virus.

Nine (2.6%) of the sera from Suffolk County residents had demonstrable CV neutralizing antibodies, but no HAI titers. One of the CV reactors also showed EE neutralization and had a reproducible HAI titer of 20 for EE. Four additional sera showed only low EE titers of 10-20. No WE antibodies were found by either test. Group B activity was somewhat more frequent than Group A. Five sera neutralized SLE virus and the SLE HAI titers for 2 were high, >320 and 80, the others 20. Two of these sera also neutralized to some extent POW virus and also POW HAI titers for these sera were 40 and 10. Four additional sera had reproducible SLE titers of 10-20, but no neutralizing ability for the two Group B viruses was noted.

Survey of bovine sera from St. Lawrence County:

Five hundred and four sera from 21 dairy herds, comprising more than 947 cows, were examined by HAI and suckling mouse neutralization tests. The farms studied were from 11 townships extending from west to east across St. Lawrence County, which is in the northern part of New York State. The blood samples were made available to us through the

courtesy of Dr. Grant S. Kaley, Director, Division of Animal Industry, New York State Department of Agriculture and Markets, and Dr. William F. Haenel and Dr. Donald Bixby, St. Lawrence County, also of the Division of Animal Industry.

Neutralization tests with CV virus were done on all sera, but neutralization tests with Group A and Group B viruses were only performed on the sera which reacted in HAI tests.

Hemagglutination-inhibition reagents were prepared as previously described. Acetone was substituted for 25% kaolin in the treatment of all sera. The sera were then adsorbed with packed goose erythrocytes. The actual dilutions and dispensing of antigens and red blood cells were done by the microtechnic (modified from Takatsy) as described by John Louis Sever (J. Immunology, 1962, 88: 320-329). Disposable V plates were used throughout.

Results: Cache Valley neutralizing antibodies were found in 109 (21.6%) of the sera. Monotypic reactions were noted for 94 sera (86.2%). Only one serum failed to show protection, and 36 sera inhibited hemagglutinating CV antigen. Multiple responses were found in 15 sera (13.8%). All 21 farms were represented but the number of reactions varied from 1 (3.3%) on Farm #1 in Gouverneur Township, a western area, to 20 or 50% on Farm #18 in Brasher Township in the eastern part of the county.

Group A antibodies were less frequently encountered. Fifty-seven (11.9%) sera had WE antibodies. Twenty-nine sera had a monotypic response. Multiple antibodies were demonstrated in 28 sera. Cross reactivity with EE was noted in 16 instances either by neutralization and/or HAI technics. Only one serum showed monotypic activity with EE when neutralizing antibodies were found.

Group B antibodies were seldom found. Nineteen (3.7%) sera representing 7 townships showed SLE activity. Eleven sera gave monotypic response and 9 had multiple antibodies. In only one serum were neutralizing antibodies detected but all 19 had reproducible HI titers of 20. Four sera (0.7%) showed neutralizing potency for POW but only one of them had an HAI titer; 3 sera also cross-reacted with SLE.

When the results were analyzed in relation to age of cows, the older the animal the higher was the percentage of reactivity noted. The age groups studied ranged from 2-10 years although 4 sera were obtained from cows 12, 13, and 17 years old. The samples were too small to be statistically valid. The percentages of neutralizing reactivity in sera for CV and WE viruses was 17.6% and 11.7%, respectively, in the 2-year-old group. Cache Valley neutralizing antibody rose to 30.3% of the 8-year-old group, leveling off to 26.3% and 28.5% of the 9- and 10-year-old animals. There were two peaks of neutralizing activity for WE virus, one in the 7-year-old and the second in the 10-year-old groups, 21.9% and 28.5%, respectively. These facts confirm the findings of Kokernot et al. (Ann. Trop. Med. and Paras., 1961, 55:73-85).

REPORT FROM DR. DELPHINE H. CLARKE
ROCKEFELLER FOUNDATION VIRUS LABORATORIES
NEW YORK, N.Y.

Ethiopian Yellow Fever Strains, their antigenic comparison with those from West Africa and South America:

An extensive outbreak of yellow fever occurred in Ethiopia beginning in 1959; although the disease became quiescent in 1960, it recurred as a more serious epidemic in 1961 and was not stabilized until the end of 1962. The disease picture showed certain distinctive clinical features, among which were a meningoencephalitis syndrome and frequent hyperacute cases. Strains of the virus were obtained for study through the courtesy of Dr. Ch. Serie, Director of the Pasteur Institute in Addis Ababa.

The present report deals with a study in progress in which the antigenic structure of Ethiopian strains is being compared with that of West African and South American strains.

The viruses being used in this study are all human isolates and are the following: 1) West African prototype - Asibi - 1927; 2) South American prototype - Be H70 - Brazil - 1954; 3) Ethiopian strain Couma - 1961; 4) Ethiopian strain Coure - 1961; 5) Ethiopian strain Manera 338 - 1962; 6) Ethiopian strain Manera 341 - 1962.

A preliminary specificity test in mice showed all four Ethiopian strains to be neutralized to essentially the same degree by an Asibi immune monkey serum as was the homologous Asibi virus. By HI, using both Asibi and Be H70 immune mouse sera, the four Ethiopian strains also reacted in a manner similar to the homologous antigens (Table 1). The converse situation, in which Ethiopian strain immune mouse sera were tested against all six antigens, was not generally remarkable except that with Coure serum, the homologous titer was significantly higher than were any of the heterologous (Table 1).

Further studies have been and are being done by the method of absorption HI which had been used previously to demonstrate that West African strains of yellow fever could be distinguished from those from South America (Trinidad and Brazil). The technique as now developed uses constant volumes of serum absorbed with the pellets obtained by high speed centrifugation of increasing volumes of a suspension of infected suckling mouse brain. The control and absorbed serum samples are tested by HI against: 1) the antigen homologous to the serum; 2) the antigen corresponding to the absorbing virus. Comparison is based on the presence or absence of a significant degree of deviation between the two curves.

Examples of the results so far obtained are shown in the two figures. In figure 1, West African Asibi strain serum was absorbed with Ethiopian strain Couma virus and Ethiopian strain Couma serum was absorbed with Asibi virus and with South American Be H70 virus. It is clear that no significant difference could be shown when Asibi serum was absorbed by the Ethiopian strain virus while a significant difference was apparent in the converse direction. A quite marked difference was observed when the Couma serum was absorbed with the South American virus.

In figure 2, Ethiopian strain Manera 338 was compared with the West African and South American prototypes. Again no difference was observed when Asibi serum was

absorbed with the Ethiopian virus whereas a clear deviation was apparent in the converse direction. Cross comparison between Manera 338 and the South American prototype Be H70 revealed differences detectable in both directions, but this was quite dramatic when the Ethiopian strain serum was absorbed with the South American virus.

Results so far obtained with the other two Ethiopian strains are in general agreement with those presented although the Coure strain appears to have certain peculiarities of its own that are not presently understood.

In the report published in 1960, evidence was presented for the presence in West African strains of yellow fever of an antigen (or antigens) lacking in those from South America. Now in the current studies on Ethiopian strains, there appears to be evidence for the presence of an antigen (or antigens) either deficient or lacking in West African strains and definitely lacking in South American ones. The nature of the converse relationship between South American and Ethiopian strains remains to be explored.

The overall picture emerging from our studies on yellow fever strains so far is that as one moves from West to East--from South America to West Africa to East Africa--the strains encountered appear to have an increasing degree of antigenic complexity. The significance of this observation is obviously speculative, but it would suggest that an extension of the work to include strains from other parts of Africa is desirable.

Table 1

Cross HI Titers of Yellow Fever Strains from West Africa, South America and Ethiopia

Sera	Antigens					
	Asibi	Be H70	Couma	Coure	Manera 338	Manera 341
Asibi	(1280)	1280	640	1280	1280	1280
Be H70	640	(1280)	640	1280	640	1280
Couma	2560	2560	(2560)	2560	5120	5120
Coure	320	320	160	(1280)	320	320
Manera 338	1280	640	1280	640	(1280)	2560
Manera 341	640	640	1280	640	1280	(2560)

Reciprocal of serum titer with 4-8 antigen units.

Figure 1

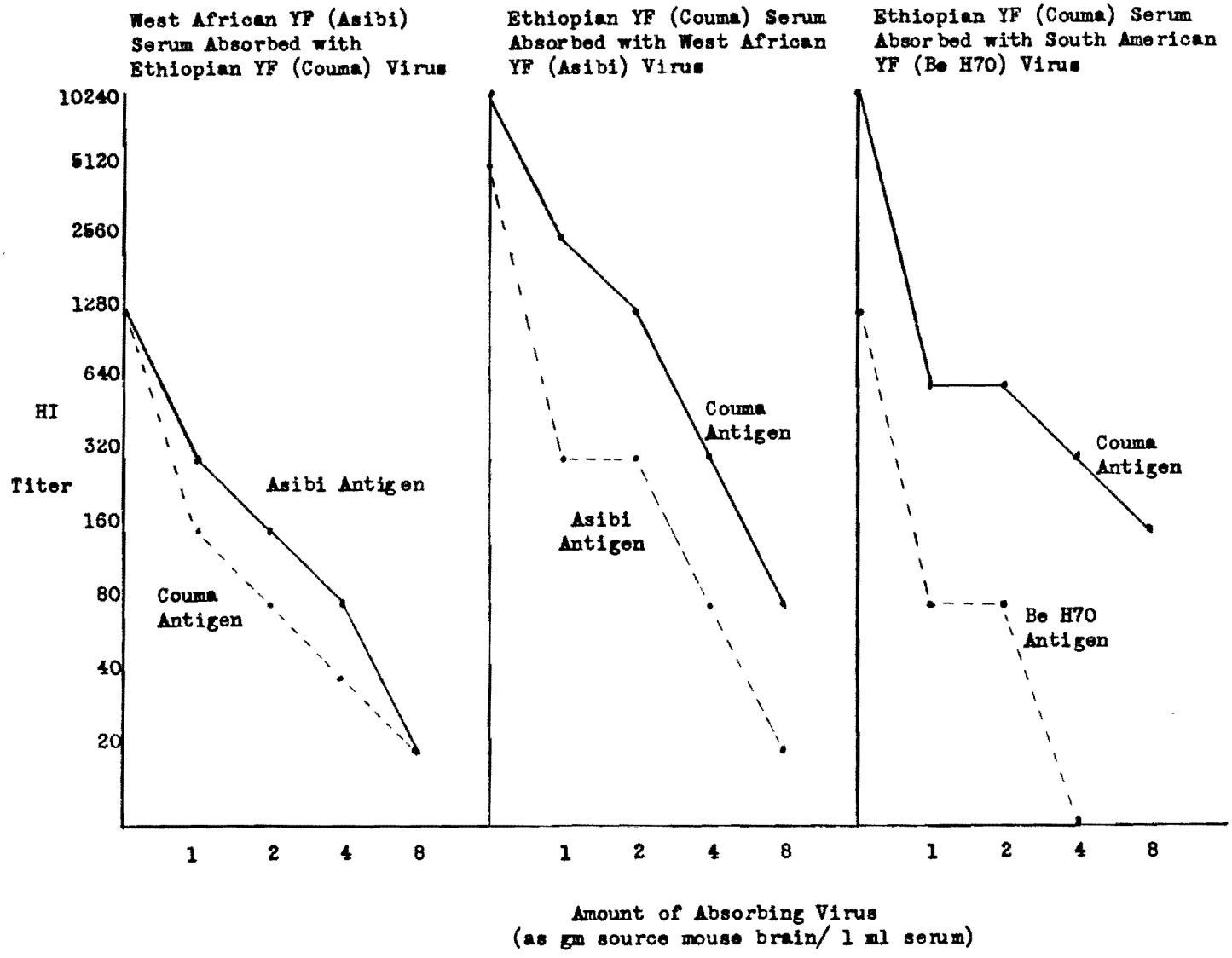
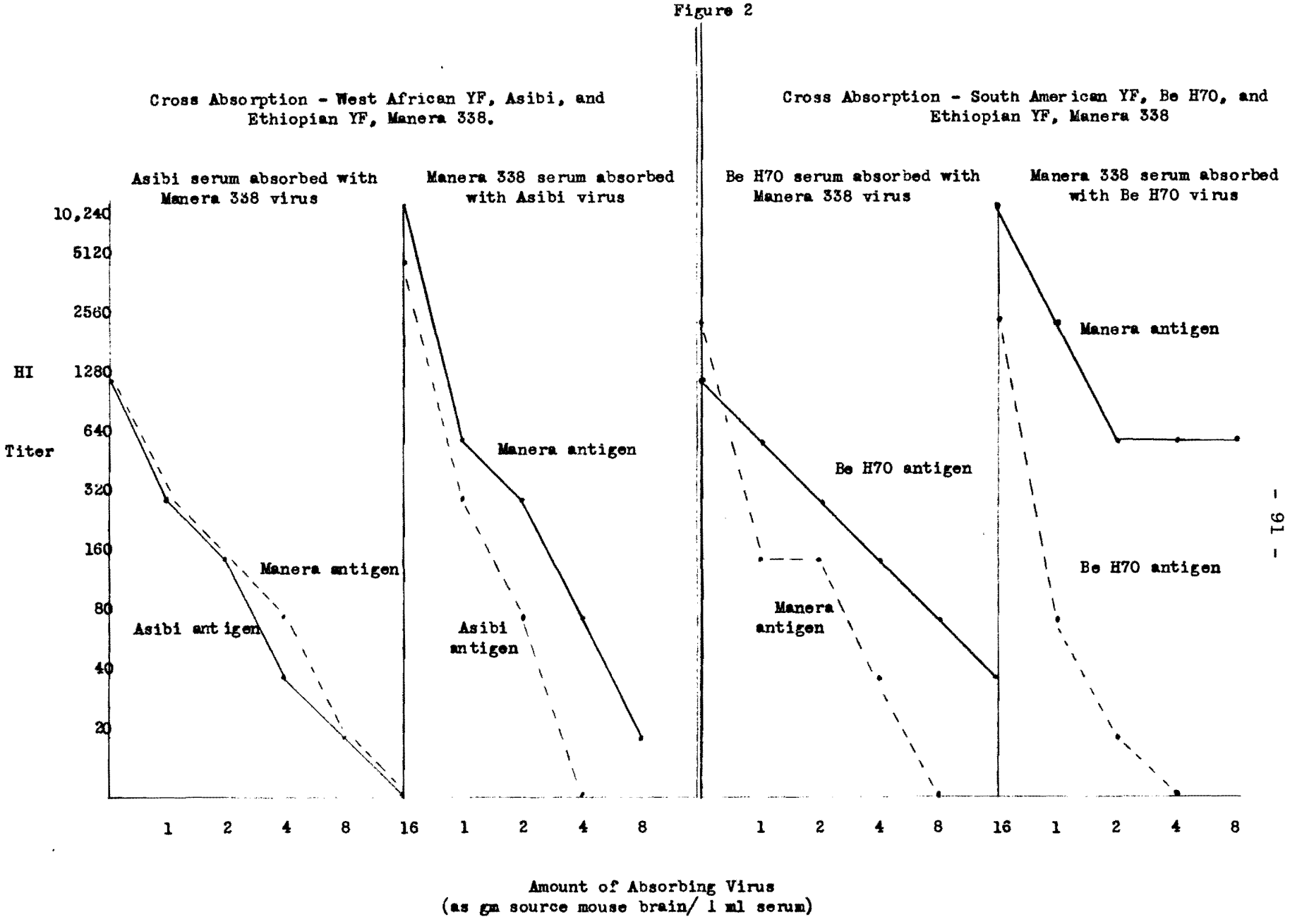

West African YF (Asibi) Serum Absorbed with Ethiopian YF (Couma) Virus
Ethiopian YF (Couma) Serum Absorbed with West African YF (Asibi) Virus
Ethiopian YF (Couma) Serum Absorbed with South American YF (Be H70) Virus
10240
5120
2560
1280
640
320
160
80
40
20
HI Titer
Asibi Antigen
Couma Antigen
Couma Antigen
Asibi Antigen
Couma Antigen
Be H70 Antigen
1
2
4
8
Amount of Absorbing Virus
(as gm source mouse brain/ 1 ml serum)

Figure 2


Cross Absorption - West African YF, Asibi, and Ethiopian YF, Manera 338.
Asibi serum absorbed with Manera 338 virus
Manera 338 serum absorbed with Asibi virus
Cross Absorption - South American YF, Be H70, and Ethiopian YF, Manera 338
Be H70 serum absorbed with Manera 338 virus
Manera 338 serum absorbed with Be H70 virus
HI Titer
10,240
5120
2560
1280
640
320
160
80
40
20
Manera antigen
Asibi antigen
Manera antigen
Asibi antigen
Be H70 antigen
Manera antigen
Manera antigen
Be H70 antigen
1 2 4 8 16 1 2 4 8
1 2 4 8 16 1 2 4 8
Amount of Absorbing Virus
(as gm source mouse brain/ 1 ml serum)

REPORT FROM DR. WILLIAM F. SCHERER
DEPARTMENT OF MICROBIOLOGY
CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK

Investigations of arthropod-borne viruses on the eastern coastal tropics of Mexico were begun during summer 1961 and continued in 1963 in collaboration with the Pan American Health Organization, the Mexican Government, and Mexican scientists (Drs. Campillo, de Mucha, and associates of the National Institute of Virology, and Dr. Diaz Najera of the Institute of Health and Tropical Diseases). Three viruses recovered from mosquitoes collected in 1961 near Tlacotalpan in the State of Veracruz were identified by complement fixation and neutralization tests as members of the Bunyamwera group, probably closely related to Cache Valley and Tensaw viruses. These viruses were from Anopheles and Mansonia collected during July and August in a pig-baited Magoon trap located in savannah land on the banks of a large river. One strain of virus, 61D240, from Mansonia titillans was successfully reisolated from frozen mosquito suspension 10 months after the original isolation, and has been chosen as the prototype virus. These viruses were inactivated by sodium deoxycholate in saline and in serum, were pathogenic for weanling mice inoculated intracranially but were not pathogenic after intraperitoneal inoculation; they produced no consistent cell destruction in hamster kidney cell cultures.

Studies of migratory birds as possible transporters of arboviruses over long distances in North America were expanded during 1963 at Tlacotalpan. Bloods from 323 migratory birds collected during March and April were negative for arboviruses upon inoculation of suckling mice; 11 bloods are still under study. During September, pools of heart, kidney, and lung tissues, as well as bloods were collected from 195 migratory birds. Surveillance of virus activity was continued during the summer by collection of 330 pools representing 12,594 mosquitoes; plasmas were obtained from sentinel chickens in the spring and fall.

During the summer of 1963, studies were also carried out approximately 75 miles south of Tlacotalpan in and near a small range of volcanic mountains which rise to 6000 feet above sea level. One study area (Arroya Agrio) was at the northern end of a lake (Catemaco) situated in these mountains at approximately 1000 feet above sea level; the other study area (Sontecomapan) was located essentially

at sea level on a lagoon approximately five miles from the Gulf of Mexico coast and approximately ten miles northeast of Arroya Agrio. Studies were carried out in the village of Sontecomapan, in a small patch of rain forest about 1200 meters from the village and in low secondary forest adjacent to bamboo and mangrove which bordered the lagoon. Collections in these two areas included mosquitoes (673 pools, 15,846 mosquitoes), birds (227 permanent residents and 34 migrants), and small mammals (191). A Bunyamwera group virus (related to 61D240 virus recovered from mosquitoes in 1961 at Tlacotalpan) was isolated from 100 female Culex iolambdis collected at Sontecomapan. An unidentified virus was also recovered from a pool of 175 female Culex spp., and Venezuelan encephalitis virus was recovered from a sentinel hamster found ill on 29 July 1963 in a mosquito trap in the rain forest near Sontecomapan. This strain of VE virus, 63U2, reacted strongly by CF test with VE antiserum and to lesser degrees with Mucambo and Pixuna antiserum (kindly supplied by Dr. J. Casals). It was neutralized by antisera representing VE strains from Trinidad, Colombia, Panama, and the vaccine TC-83 attenuated virus (kindly supplied by Major R. McKinney). One natural VE viral infection occurred in personnel working at Sontecomapan during August and a laboratory infection occurred in New York during October. Both were characterized by fever, chills, generalized malaise and aching, recovery of a suckling mouse pathogenic agent from acute-phase blood and development of CF antibodies to 63U2 virus; no symptoms of encephalitic, gastrointestinal or respiratory disease occurred.

Nodamura virus (formerly Mag 115) continued to remain unidentified. Swine infection was studied further (in collaboration with Dr. Richard Shope) by subcutaneous inoculation of a pig with Nodamura virus; no viremia could be demonstrated, but specific neutralizing antibodies developed. Neutralization or CF tests failed to show relationships with hog cholera, Teschen disease, or mouse hepatitis viruses.

Continued attempts to identify two unclassified viruses recovered from birds in Japan during 1954 (Tsuruse and K622) failed to show relationships by CF tests with mouse hepatitis viruses.

As part of the activities of the Subcommittee on Serologic Reagents of the American Committee on Arthropod-borne Viruses, the procedure for production of antibodies in

plasmas and ascitic fluids of mice utilizing complete Freund adjuvant was tried with Naples sandfly fever virus. A strain in 7th or 9th suckling mouse passage from human blood was used. CF antibody titers were equal in plasmas and ascitic fluids, and antibodies prepared against virus passed at low or at high (limiting) dilutions of brain reacted equally well with CF antigens made from virus passed either at low dilutions or at high dilutions.

REPORT FROM DRS. MARTIN GOLDFIELD AND OSCAR SUSSMAN NEW JERSEY DEPARTMENT OF HEALTH, TRENTON, N.J.

Enzootic studies of EE and WE have been proceeding in a coordinated way at three previously described field sites in New Jersey's EE epidemic area and at one control site for the fourth consecutive year. Progress in this rather large-scale study will, of necessity, be outlined in rather terse fashion below.

Humans: A surveillance system for encephalitis covering the state is continuing. Suspicious cases of encephalitis are reported, materials collected and studied. In fatal cases, whole brains are submitted for both pathologic and virologic studies. EE cases and inapparent infections surviving the '59 outbreak have been bled sequentially for four successive years and serial CF, HI, and MNI titers have been performed. Fifth year bleedings are being collected at the present time. Careful neurologic follow-up of cases and inapparent infections that occurred in 1959 continues with residuals detectable in both groups. No epidemic arbovirus activity has been noted since 1959.

Horses and Pheasant Flocks: Surveillance of disease in horses and pheasant flocks continues, with the cooperation of all veterinarians in the state and other departments of state government. Since no information is available on vaccination status of horses, this activity is of unknown value at the present time.

Wild Birds: Approximately 8,000 birds were netted, banded, and bled between January 1 and December 31, 1963. All have been studied for viremia by inoculation of chicks. Twenty-two isolations of WE and four of EE were obtained, with bird infections detected no earlier than June 30th and as late as December 11th. Two hundred eighty wild birds found dead in nets were studied for arbovirus infection of brain and spleen with completely negative results.

Approximately 1,500 birds were netted, banded, bled, and studied between January 1 and mid-June, 1964, with no yield of arbovirus and examination of an additional 152 birds found dead in nets also proved negative. Failure to detect avian infections during the period of mid-December to the present contrasts sharply with results of non-avian studies during the same time-period reported below.

Arthropods: More than 70,000 mosquitoes were obtained in field collections at the four study sites during 1963. Thus far, 711 pools, (approximately 60%) of mosquitoes have been studied with a yield of only two isolations of WE from C. melanura mosquitoes. Speciation of the remainder is 90% complete; pooling and testing should be accomplished in the very near future.

About 29,000 mosquitoes have been trapped in 1964 as of early July. Eighty-seven pools containing 4,000 mosquitoes have been studied thus far with no arbovirus yield. All blooded mosquitoes are being studied by microprecipitin techniques in cooperation with Wayne Crans, Rutgers University, and pooled by mosquito species and variety of blood meal ingested prior to virus isolation attempts.

More than 5,000 non-avian vertebrate ectoparasites and 1,000 ticks were collected in 1963, and close to 3,000 additional ectoparasites have been obtained in the first half of 1964. Of these, two pools of mouse fleas and one of mouse lice collected in January, January, and March of 1964, respectively, yielded WE isolates. Two of the three were successfully reisolated.

Miscellaneous Vertebrates: As of January 1, 1964, 2,105 non-avian vertebrates had been trapped and brought to the laboratory. Brain was studied for arbovirus presence in all, and bleedings were accomplished in 1,219. Bloods were examined by virus isolation techniques and for EE and/or WE neutralizing activity in wet chicks. In addition, 683 bleedings were obtained from vertebrates in the field and similarly examined. During the first half of 1964, 755 additional vertebrates have been brought to the laboratory and 394 additional bleedings were obtained in the field. In summary, about 4,000 miscellaneous vertebrates of some 60 species have been examined to date.

As of June, 1964, seven isolates of EE and 26 of WE have been obtained, 22 of which have been successfully re-isolated thus far. As seen in Table 1, isolates have been obtained from five varieties of rodents and three varieties of turtles, as well as deer, red fox, raccoon, and spring peeper. Interestingly, nine of the twelve indicated species have also revealed evidence of EE and/or WE neutralizing activity in serologic studies to be outlined below. Isolations from these species have been made in September, November, and December of 1962, February, April, August, September, October, November, and December of 1963 and January, February, March, April, and May of 1964. With the exception of one strain of WE obtained from the blood of a Northern Diamondbacked Terrapin, no isolations were made from animals trapped in the months of June, July, or August. Moreover, viremia has thus far not been detected in non-avian vertebrates during the period between May 1st and October 1st, with the single exception of the turtle detailed above.

EE neutralization indices were determined in wet chicks for 1,633 non-avian vertebrate bleedings as of January 1, 1964, and WE CNI's were obtained on 1,790 samples. Conservative criteria for interpretation of results were adopted as follows:

1) A result of 2.0 logs or more was considered positive.

2) A result of 1.8 or 1.9 logs was considered positive when repeat testing in mice showed similar results.

3) A single result of 1.8 or 1.9 logs was considered positive in whole bloods when they were already diluted 1:10 or more prior to testing.

By these rigid criteria, significant EE or WE neutralizing activity was found in samples of 22 species among the 60 tested, as illustrated in Table 2. Ten species revealed positive specimens for both EE and WE, ten for WE only and two for EE only. Nine of the 22 species have yielded EE or WE virus isolations from brain and/or blood.

In summary, serologic studies have suggested the involvement of a number of non-avian vertebrate species in the

enzootic cycling of EE and WE in New Jersey. Isolation of these agents from such vertebrates has been accomplished in every month of the period August, 1963, through May, 1964. It appears, therefore, that the first clear evidence is in the process of developing that EE and WE survive throughout the year in this state. Studies of mammalian ectoparasites have yielded two isolates of WE from fleas and one from lice obtained from white-footed mice trapped in colder months of the year. Although these results will require confirmation by additional work, they at least suggest a possibly fruitful avenue in the search for a year-round non-avian vector.

TABLE 1

ISOLATIONS OF EE AND WE FROM NON-AVIAN VERTEBRATES

ANIMAL SPECIES	No. of Isolates			
	Brain		Blood	
	EE	WE	EE	WE
Whitetail Deer*	-	-	1	-
Red Fox*	-	-	-	2
Raccoon*	-	-	-	1
Norway Rat*	-	1	-	5
Eastern Cottontail*	-	1	1	2
Meadow Vole*	-	-	-	1
White-faced Mouse*	2	5	2	4
Pine Vole***	-	1	-	-
No. Diamondback Terrapin*	-	-	-	1
E. Box Turtle*	-	-	-	1
Snapping Turtle	1	-	-	-
Spring Peeper**	-	-	-	1
All	3	8	4	18

* Positive neut. antibody demonstrated in some specimens
** No blood ever tested for neut. antibody
*** Only 3 specimens tested for WE neut. antibody

TABLE 2

EE AND WE NEUTRALIZATION TESTS
NON-AVIAN VERTEBRATES

ANIMAL SPECIES	EE NEUTS		WE NEUTS	
	No. Tested	No. Pos.	No. Tested	No. Pos.
Horse	6	2	6	3
Goat	3	1	3	0
Whitetail Deer	27	1	27	2
Fox, unspecified	-	-	3	1
Red fox	10	0	10	1
Raccoon	41	3	46	1
Norway rat	29	2	29	0
Eastern Cottontail	108	1	108	2
Meadow Vole	43	1	53	3
Eastern Harvest Mouse	7	0	7	1
White-footed Mouse	894	11	1020	47
House Mouse	28	0	28	2
No. Diamondback Terrapin	31	1	31	1
Stinkpot	3	1	3	1
Eastern Box Turtle	106	1	94	3
Spotted Turtle	6	0	6	1
Eastern Painted Turlte	53	0	53	1
Bullfrog	4	3	14	3
So. Leopard Frog	2	0	8	2
American toad	26	0	26	3
Black rat snake	6	0	7	1
Rough green snake	-	-	1	1

REPORT FROM DR. W.McD. HAMMON
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UNIVERSITY OF PITTSBURGH GRADUATE SCHOOL OF PUBLIC HEALTH

I. CALIFORNIA ENCEPHALITIS VIRUS AND OTHER MEMBERS OF GROUP OR COMPLEX

Electron Microscopy: Size studies which we made on California encephalitis virus (CEV) in 1945 using gradacol type membranes indicated a size between 60 and 125 mμ. At that time this seemed unexpectedly large for an arbovirus. Cultures have recently been grown in hamster kidney tissue culture (HKTC) and examined by Dr. Atchison in drops of partially purified fluid and in tissue culture cell pellet sections by electronmicroscopy. This study is incomplete but the virus appears to be spherical or polyhedral with dense core and a surrounding membrane, and most particles measure about 50 mμ with range from 49 to 62 by both methods. Virus particles are not found in the nucleus but are found fully formed in the cytoplasm, in largest numbers just outside the nuclear membrane.

Florida Serological Survey for CEV and Failure of Acetone to Extract Inhibitor: From Hillsborough County, Florida, 102 normal survey sera sent by the Regional Encephalitis Center, Tampa, were tested against CEV (BFS-283) antigen by the HI test. Six were positive at a 1:10 or a 1:20 level. Three of these were checked for specificity by the neutralization test and all had NI's of $\geq$ 2.5 logs. Three negative by HI were negative by neutralization. This alerted to presence of a related agent in the area.

Antigen prepared here was then sent to Tampa and used to test a group of selected sera by HAI and to our astonishment about 60% were positive. On testing 19 of the positives in Pittsburgh by neutralization,only one was positive, and subsequently retesting all 19 positives here by HI with the same antigen and two other lots of BFS-283 antigen negative results were obtained with all. Comparison of methods revealed that in Tampa acetone extraction was used and kaolin was used here. A comparison was then made here with kaolin and acetone extraction with 62 fresh sera from the original group. Nine other antigens from groups A, B, C and Bunyamwera were included. Twelve sera were positive to CEV when acetone extracted but not one when kaolin was used, and only one of these positives reacted with a single Bunyamwera group and two with a single group B virus, showing quite conclusively that non specific inhibitor for CEV virus is frequently not removed by acetone.

Identification of Eight California-related Viruses Isolated from Florida Mosquitoes: Eight viruses isolated in suckling mice in 1963 from Aedes infirmatus by the Tampa Regional Encephalitis Center were sent to us for identification after shown locally to be DCA susceptible, without ability to hemagglutinate, in general not pathogenic for weanling mice and antigenically apparently not members of group A, B or Bunyamwera (only groups previously recognized in area by virus isolation and identification). These were all identified in Pittsburgh as members of the California group and all appeared to be immunologically closely related to the prototype we selected from the group, B63-373B. Comparisons made with CEV and trivitattus viruses by neutralization, CF and HI (one direction only possible by HI) showed them to be more closely related to trivitattus than to CEV. They may be trivitattus, but minor differences, at least, are apparent and this is under extensive study.

Encephalitis Case With CEV Antibody Conversion: On 8/22/63 a 12-year-old female in the Tampa area became ill with a severe encephalitis associated with coma. Serial serum specimens were submitted to the Tampa Research Encephalitis Center. HI tests against EEE, WEE, SLE and Tensaw antigens were negative as well as tests for leptospira and mumps. No attempt was made to recover a virus. The HI test using CEV antigen (BFS-283) showed a serological rise from 1:10 to 1:160 in 29 days. The serum series, including a serum specimen collected 110 days after onset, were sent to this laboratory for further testing. The HI test was repeated and the results confirmed. CF and neutralization tests with several viruses in the group were performed as shown below.

SEROLOGICAL RESULTS - FLORIDA ENCEPHALITIS CASE, S.H.

Days after onset	HI		CF			Neutralization index		
	CEV*	SLE	CEV	Triv.†	B63-373B‡	CEV	Triv.	B63-373B
1	<10	<10	0	0	0	<0.5	<0.6	0.73
15	80	<10	0	0	0	3.0	2.3	1.82
29	640	<10	16	0	8	3.5	3.3	2.63
110	20	<10	32	4	8	2.8	3.3	3.30

* California Encephalitis Virus - 3 separate lots of antigens used and results did not differ by more than 1 tube

† Trivittatus virus of Eklund

‡ B63-373B isolate from Florida A. infirmatus which is more closely related to Trivittatus than to CEV

Though not conclusive, this is strong evidence that a member of the California group produced the disease. CF results which are more specific than other tests in distinguishing members of this group suggest that a CEV-like virus, not B63-373B, was responsible. Previously we had shown titer rises to CEV in three encephalitis cases in California although one of these cases also had a SLE rise. Until the current case no other cases have been found in the United States which could be attributable to members of the California virus group despite the inclusion of this virus in diagnostic tests done on several hundred cases in California. Possibly a second case has been found among the diagnostic specimens submitted to the Jacksonville laboratory, Florida State Board of Health. Using the CEV antigen in the CF test this person had a serological rise from <1:4 to >1:64 in two specimens 14 days apart. No details are known to us of the illness.

Comparison of CEV and Related Strains Isolated in the United States and Other Countries: BFS-283, BFS-91 and BFS-395 (our original isolates from mosquitoes in California), trivitattus of Eklund from Montana, the San Angelo virus from Anopheles pseudopunctipennis isolated by Grimes, Garza and Irons from Texas, snowshoe hare isolate by Burgdorfer, et al., from Montana, Buttonwillow virus, BFS-4474 and BFS-4470 isolated by Reeves from California mosquitoes, plus Tahyna, Melao, Lumbo and Guaroa are all being compared by all possible methods to supplement studies by Whitman and Shope. Results are still very incomplete but at present we are able to confirm that all those now in the virus catalogue are apparently readily differentiated one from the other, and most readily by CF tests. Buttonwillow virus, however, shows no relationship to the group. HA antigens of useable titer have been prepared from BFS-283, BFS-91, snowshoe hare and Tahyna,

and low titered antigens which can be used at a 4-unit level from Buttonwillow and one Florida strain. Strains believed to be in this group, isolated in the United States, are requested for inclusion. Promises of up to a year ago from some who have these strains have still not been activated.

II. LIVE ATTENUATED JBE VIRUS VACCINE FROM STRAIN OCT-541

24°C Line in Human Volunteers: Twelve terminal cancer patients in Pittsburgh and 12 more at the Roswell Park Memorial Hospital in Buffalo have received the live virus. The last group received 1 to 2 ml of material titering $10^{6.0}$ to $10^{6.5}$ per ml. None but the first two in the Pittsburgh series have any response suggesting even infection. The last to be injected received a second dose three months after the first and a significant neutralizing antibody response occurred in the TC test but an equivocal level by the mouse test. Thus, this live virus is apparently reasonably safe but has no apparent advantage over an inactivated one.

37° Line: One line derived at this temperature and found to have certain more favorable attributes has now been discarded after comparative antigenic studies. It has undergone an undesirable antigenic change and no longer produces good antibody or protection against several wild or classical strains.

III. FORMALIN INACTIVATED JBE TISSUE CULTURE VACCINE FROM 24°C ATTENUATED LINE OF OCT-541

Progress is still very satisfactory and many minor variations developed by Dr. Darwish have led to attainment of higher infectious and HA titers prior to inactivation and better MID's after inactivation. Lots are now in preparation or undergoing safety trials for human testing. A monkey potency test is showing promise.

IV. TISSUE CULTURE AND PLAQUE STUDIES OF JBE VIRUS STRAINS

Dr. Katsuji Nagai, a postdoctoral fellow, has now completed his studies and a series of papers has been written with the first now in press (Proc. Soc. Exper. Biol. & Med.). They deal with comparisons of several JBE strains, plaque characteristics in HKTC and CETC, hamster liver cell, lung cell, embryo cell and cerebellum cultures. Mouse lung and cerebellum were also tested. Secondary cells were tested for some cell culture types.

V. ELECTRON MICROSCOPY OF JBE VIRUS

Electron microscopy was done for JBE virus (OCT-541 24°C line) on purified virus pellet and on sections of infected hamster kidney tissue culture by Dr. Atchison. The virus particles were spherical with capsid showing capsomere structure. Some empty particles were seen. The size for the pellet particles was 42 - 56 mμ whereas in the tissue culture sections it was 33 - 46 mμ.

VI. ST. LOUIS VIRUS STUDIES

Selection of Strains for Serological Work in Florida: A number of strains tentatively or partially identified as SLE viruses from the Tampa Bay area of Florida were sent to us for study. Several were completely identified including tests by cross vaccination-challenge. Several along with older classical strains

were compared to select the most appropriate for use as HI and CF antigens for local Florida survey and diagnostic work and four were tested for plaque forming ability and their possible use for plaque neutralization tests.

For HI tests the several local strains appeared to be essentially the same and gave higher titers with local sera than did the Webster strain. Pinellas-15 (first mosquito isolate from Tampa Bay area 1961) and TBH-28 (brain isolate of 1962) were compared in many tests for a variety of properties and were found to be essentially identical even to plaque forming ability and heterogeneity of plaques. Either appeared to be well suited to any application for serological work in that area.

Plaques for SLE Serology: Large and small plaques were obtained from Pinellas-15 and TBH-28. Serial plaque picking was employed to clone the former and differential tests indicated both clones were antigenically similar but that they differed significantly in mouse and tissue culture pathogenicity. The large plaque proved advantageous in plaque neutralization tests and many sera from Florida patients and "normals" were tested in parallel with the standard mouse i.c. test. Complete correlation occurred with mouse positive sera using undiluted serum for both with 1.7 logs for mouse N.I. and 67% plaque reduction as criteria for positive. The plaque test gave additional positives. Normal human serum heated or unheated may give up to about 50% plaque reduction while 1:4 serum seldom shows significant suppression. Limits of sensitivity and specificity at various dilutions remain to be determined, but presently we feel that a 1:4 serum dilution is to be prefered. Chicken sera (normal) even at a 1:4 or 1:8 dilution give non specific suppression of plaques.

Significance of HI for SLE in Tampa Bay Area: Florida sera from laboratory confirmed cases of encephalitis as in our past experience in Western states are found to have neutralizing antibodies during convalescence and for several years subsequently with great regularity. In normal numan survey sera of this area, well over 50% of sera with SLE HI titers of 1:10 to 1:80 (<1:10 for EEE, WEE and Tensaw) are found to be without significant levels of SLE neutralizing antibodies by the i.c. mouse test. The nature of this HI inhibitor is now under study. Is it group B specific inhibitor, due to a virus or viruses other than SLE, but not yet isolated, or is it a group B inhibitor not resulting from group B infection but not removed by acetone, in a manner similar to the failure of acetone to remove inhibitors for CEV virus HA antigen?

VII. TRINIDAD VIRUS ISOLATE

We reported (Newsletter #8) a virus isolation from serum of a patient apparently infected in Trinidad. This has proved to be another virus in the mouse hepatitis group, possibly acquired by the inoculated mice from their environment.

VIII. PHILIPPINE ISLANDS STUDY OF 1956 - PAIRED SERA OF FEBRILE SCHOOL CHILDREN

Results of this study were reported in part in Information Exchange #8. Further results are as follows. No viruses were isolated from acute phase sera of ten group B converters. Fifty-six percent of the 173 school children without titer change had HI antibody to dengue 3 (our most common isolate from Manila) and a high percentage of these also had antibody against JBE, MVE, TP-21 and Ntaya (only other group B antigens used). An occasional one was positive to JBE and not dengue; a few more were positive at a 1:10 to 1:20 level for MVE only. A few of the low

titered MVE positives were tested by mouse neutralization test against several group B viruses and it appears that the virus responsible is not MVE. Four persons with TP-21 HI titers of 10-320 were negative to TP-21 virus by mouse neutralization tests, but they had mouse NI's of 2.4 to 3.5 logs against dengue type 2. No positives were found against CEV.

IX. DENGUE AND HEMORRHAGIC FEVER OF SOUTHEAST ASIA

Dengue Monkey Typing Sera: Rhesus monkeys (2 for each virus type) were given a single large subcutaneous injection of terminal dilution purified dengue viruses and antibody development (HI, CF and NI) followed at short intervals for 177 to 261 days. Homologous neutralizing antibody (suckling mouse test) developed gradually, generally appearing in 21-30 days and reaching a peak or plateau around 90 days. A great deal of variation occurred from animal to animal and between types. Heterologous neutralizing antibody to a closely related type tended to develop at a slower rate and generally did not reach the same level as the homologous antibody. Late sera, however, appeared to be less type specific than earlier ones. HI and CF antibodies were disappointing in many instances. Generally, CF antibodies were not formed, or were at a low level and disappeared very rapidly. HI antibodies occasionally appeared earlier than neutralizing antibodies (13-20 days). Some persisted for the period of observation while others fell rapidly and some disappeared completely. Some were completely group specific rather than type specific, while some were quite type specific. The level of antibody, the degree of specificity, and the duration of antibody were apparently functions of the virus type and the animal and possibly also of the virus strain.

Identification of Additional Dengue Strains from Bangkok: Two agents isolated by Dr. Rudnick from mosquitoes collected in Bangkok in 1961 while a member of this department have been under study here for identification. By CF, one agent designated X-2 appears to be closely related to TH-Sman (dengue type 6?) or to dengue 1. The second agent, X-34, is proving difficult to adapt to suckling mice even after 13-14 mouse passages. Signs, paralysis, and death occur in 13-19 days, with illness of several days duration, using 10% mouse brain suspension. The virus is low titered. Only occasionally do mice succumb at 10^{-2} or 10^{-3} dilutions. Surviving mice are immune to challenge with dengue, Trinidad strain. In BSC-1 tissue it does not interfere with CPE of EEE challenge virus.

Six agents isolated by Major Halstead (MC, USA) at the SEATO laboratory in Bangkok are currently under study here. These have been identified at the SEATO laboratory as strains of dengue. Five of the agents were isolated from Americans with clinical dengue, not hemorrhagic fever, and one agent, BKM-60, was isolated from A. aegypti mosquitoes and is included in these studies because it appears to be a "pure" TH-36 (dengue type 5?) with minimal overlapping with dengue 2, according to Major Halstead's data. Typing and cross-comparisons with the prototypes of all strains is far from complete. One agent, 5037-62, appears to be more closely related to TH-Sman virus (type 6?) than to dengue 1 by neutralization test, using immediate intracerebral inoculation of suckling mice (SM) without incubation of the serum-virus mixture. A second agent, 5029-62, appears to be more closely related to dengue 1 than to TH-Sman by the standard SM neutralization test.

It is hoped that when these studies are completed strains will be available which will be better prototypes for dengue types 5 and 6 than the current suggested prototypes and, secondly, that strains of types 5 and 6 may be found which have produced classical clinical dengue rather than hemorrhagic fever so that human volunteer studies for development of a vaccine can be made with a greater feeling of safety.

Type Specificity of Sucrose-Acetone vs. Benzene Extracted Antigens: Initial studies in our laboratory typing dengue viruses utilized antigens prepared by the benzene extraction technique of Espana and Hammon for the CF test. Recently, sucrose-acetone extracted antigens (Clarke and Casals) have been substituted. Difficulties were encountered in reproducing some of our earlier results with previously tested sera. A comparison was made with the two types of dengue antigens and hyperimmune dengue antisera. It was found that sucrose-acetone antigens were generally of higher titer and possibly more sensitive. Higher antigen titers resulted with the homologous system. Serum titers were seldom significantly higher, either the same or sometimes one or two tubes higher. However, more specific results were obtained with the benzene extracted antigens when the optimal antigen dilution of each dengue antigen was used. For example, with two mouse immune sera prepared against TH-Sman virus (type 6?) the maximum titer was obtained with the homologous benzene extracted antigen, with detectable but lower titered crossing with types 1 and TH-36 (type 5?). However, with sucrose-acetone antigens high titers of essentially the same magnitude (128-512) were obtained with all six dengue types, making it impossible to distinguish virus type on the basis of the CF reaction.

Interference Tests in Tissue Culture: The utilization of the interference phenomenon of the dengue viruses in tissue culture was investigated further, subsequent to our work reported in 1963. This time we used the BSC-1 cell line (Cercopithecus monkey kidney) obtained from Col. Buescher and maintained in this laboratory. Initial studies utilized EEE virus for the challenge. Both interference of CPE in tube cultures and reduction of EEE virus plaques (number and size) were utilized to detect known dengue virus activity in infected cultures. Results with the two techniques were quite similar. Results with the six dengue types indicate that these viruses grow in the BSC-1 cell line, and that interference can be detected as early as day 3 but that titers more nearly approximated those observed in the suckling mouse when challenge was at day 8-10. The tube technique is more satisfactory than the plaque technique due to technical difficulties of maintaining older tissue under agar. In general, results obtained were quite similar to those reported recently by Major Halstead utilizing polio type 1 as challenge virus.

With unadapted or poorly adapted agents isolated by using suckling mice, the sensitivity of the system was variable. Of six agents tested, interference was detected with three when challenged on day 10. The three not exhibiting interference are low titered suckling mouse agents which appear to be dengue by at least one immunological test, but which have been difficult to adapt to mice. No attempt has been made to adapt these to tissue culture.

Field Trip to Calcutta, Rangoon and Bangkok, January 1964: At the request of WHO a trip was made to investigate reports of possible epidemics of hemorrhagic fever in Calcutta and Rangoon which had occurred in late 1963. Reports of other visitors and of several laboratories in relation to this appeared in the No. 9 Information Exchange and others will probably appear in this one. I will only give my interpretation of data and laboratory work collected or performed in large part by others.

Calcutta had a very severe epidemic of probably tens of thousands of cases of disease clinically resembling the hemorrhagic fevers I had observed in Manila and Bangkok. Significant mortality was observed. The unusual feature was that all ages

were involved, not just children, and involvement was not limited to Orientals. Serology and failure to isolate virus readily placed the etiology in group B, probably dengue. During this epidemic, at about the peak, a second epidemic disease of a very different nature superimposed itself. It was essentially a classical dengue-like disease with rash and prominent arthralgia and again showed no age restriction. From these cases chikungunya virus was isolated and serological response was to group A. Both diseases behaved epidemiologically like those due to newly introduced agents for which the population had no previous immunity. Calcutta, however, has been recognized as an endemic dengue area, with type 1 virus isolated during World War II, and antibody surveys carried out more recently suggested types 1 and 2 are active.

Rangoon had a severe dengue-like epidemic involving all ages and possibly 50% of the total population. This was associated with rash and arthralgia and no hemorrhage or fatalities of which I could learn. Also, I was informed that "dengue" epidemics had never been recognized previously in this city. No serology had been done. My guess is newly imported chikungunya.

There is a high probability that rapidly increasing, fast international travel from areas to the east where viruses of the dengue group causing hemorrhagic fever and chikungunya virus have been recognized has brought persons with viremia, or who subsequently developed viremia, to Calcutta and Rangoon in 1963, and this served as a source of local mosquito infection (Aedes aegypti) of which there were adequate numbersin both cities. Apparently only one virus was implanted in Rangoon and two in Calcutta. Further spread may well be expected to areas with adequate populations of capable vectors.

Jamaica Dengue Virus Isolation Attempts: Early acute phase sera from 33 febrile patients from the 1963 outbreak were received from Dr. Louis S. Grant of the University of the West Indies, Jamaica, for attempted virus isolation. These were tested in suckling mice, and in BSC-1 tissue using an interference technique. Three blind passages were made in suckling mice, and survivors were challenged with dengue, Trinidad strain. Two passages were made in BSC-1 tissue, testing for the development of interference with EEE virus. Several tissue culture suspensions were also tested in mice. No virus compatible with dengue was isolated, nor was dengue immunity demonstrated in the surviving mice. However, three pathogenic agents were isolated but have not been definitely identified. The incubation period is 3-5 days. The mice exhibit encephalitic signs, with twitching and convulsive movements but no signs of paralysis. The agents are low titered (2.5 - 3.5 logs). A "quick' antigen prepared for one of the agents at passage 5 in pH 9.0 borate buffer reacted weakly in a CF test against antisera prepared against two agents shown to be related to mouse hepatitis virus (MHV), but they did not react with dengue type 3 antiserum. Similar antigens prepared for the other two agents (at P3 and 4 level) did not react with serum to the MHV-related agents, nor to a dengue type 4 serum. Two of the agents are not DCA sensitive.

REPORT FROM DR. ARTHUR N. GORELICK
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Immunological Studies on Arboviruses*:

In his studies on the cross-protection among Group A arboviruses, Dr. W.P. Allen has shown that infection of mice or guinea pigs with any of several related viruses could educe resistance to viruses such as eastern equine encephalitis, Semliki Forest, Venezuelan equine encephalitis, or chikungunya. He has recently carried out similar studies in a small number of rhesus monkeys employing Sindbis, Semliki Forest, or Una virus as "protective" agents against infection with Venezuelan equine encephalitis. Some information was also collected on the susceptibility of rhesus monkeys to the first three viruses.

For immunization, three pairs of rhesus monkeys were inoculated intravenously each with either Sindbis virus ($10^{6.3}$ suckling mouse intracerebral LD_{50}), Una virus ($10^{7.3}$ $SMICLD_{50}$), or Semliki Forest virus ($10^{8.3}$ $SMICLD_{50}$). Only two monkeys showed abnormal elevations in temperature; both had been inoculated with Semliki Forest virus. One showed a slight elevation of night time temperature beginning 24 hours after inoculation and persisted for 5 days. The other monkey showed a slight rise in evening temperature 12 hours after inoculation, but its temperature returned to normal within 24 hours. Daily blood samples revealed no circulating virus in monkeys inoculated with Una virus and only low titers or trace amounts were detected through the second day in monkeys inoculated with Sindbis or Semliki Forest viruses. From these data, it appeared that rhesus monkeys were resistant to infection with these viruses, yet all monkeys formed significant levels of neutralizing antibody against the respective viruses by the 7th day post inoculation.

*In conducting the research reported herein, the investigator(s) adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Ten weeks after inoculation with the above viruses, the monkeys were challenged by the intradermal route with $10^{5.7}$ mouse-intracerebral-LD_{50} of Venezuelan equine encephalitis virus (Trinidad strain). One monkey that had no known previous exposure to group A viruses was similarly inoculated. For the first three days post challenge, all monkeys had viremia and no significant differences were noted between the virus titers in the bloods of the "immunized" monkeys and the one unimmunized monkey (control). All animals developed significant levels of anti-VEE virus neutralizing antibodies. All had a fever, perhaps less severe in those previously exposed to Group A viruses. Thus, rhesus monkeys administered Una, Semliki Forest, or Sindbis virus were incapable of warding off a frank infection with the relatively high dose of Venezuelan equine encephalitis virus employed for challenge.

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

Captain Herbert S. Hurlbut, MSC, USN, has returned to the Department of Microbiology, Naval Medical Research Institute, after a tour of four years at the Naval Medical Research Unit No. 2 in Taipei. He will continue his investigations of arthropod host-virus relationships.

REPORT FROM THE FLORIDA STATE BOARD OF HEALTH ENCEPHALITIS RESEARCH CENTER, TAMPA, FLORIDA

During the first six months of 1964, the most significant observations of the Encephalitis Research Center (ERC) have been related to the activity of Group A arboviruses in the Tampa Bay area. Included in this group in Florida are eastern encephalitis, western encephalitis, and the newly recognized Venezuelan encephalitis.

Nineteen clinical cases of encephalitis in horses have been reported to the center; all but two from Hillsborough County. From these, eight brains have been obtained for viral and histologic studies. In one, WE virus has been identified (by CF test); in four, a viral agent is present but as yet unidentified; and in one, no virus was isolated. The remainder of the brains are in process. Sera have been obtained on nine of the reported horse cases. Studies

with HAI antigens are completed in five; four yielded high titers to EE; a fifth has no EE antibody but WE titer of 1:40. None of the animals were vaccinated within the past year.

Special collections of mosquitoes with chick-baited, light, and truck traps have been made in areas where the horses were exposed. These collections supplemented those in thirteen routine sites in the four counties operated on a weekly basis. Of 144 pools of Culiseta melanura (2,785 mosquitoes) tested or in process, eight recoveries of virus have been made. WE has been identified in one, EE in one (both by SN test) four have a presumptive Group A arbovirus, and the remaining two at present are of unknown identity. Aedes infirmatus, a fresh flood water mosquito known to bite man, has been collected and processed in 174 pools (6,099 mosquitoes). There have been six viral isolations, three presumptively identified as belonging to the Group A and three in the California group.

Virus recoveries have also been obtained in two of 64 pools of Anopheles crucians, and one of 19 pools of Aedes atlanticus-tormentor; none of these viruses are as yet identified. A total of 1,563 pools representing 45,433 mosquitoes collected in 1964 have been tested or are in process; from these seventeen positive pools have been obtained as described above.

Serologic examination for HAI antibodies to EE, WE, SLE, and California virus have been completed on 158 humans with suspected viral CNS infection, 263 mammals, 207 wild birds, 70 sentinel chickens, and 293 amphibians or reptiles. There have been no serologic confirmations of recent arbovirus illness in humans. Four adult blue jays captured in May in St. Petersburg had serologic evidence of past infection with Group A arbovirus (EE titers high, WE titers intermediate). A dove from the same area was captured after a year (Fall 1963-May 1964) and SLE-HAI antibodies were shown to change from negative to positive titer of 1:20. Sentinel chickens established in January 1964 and bled every two months were maintained in fourteen locations throughout the three counties (Pinellas, Hillsborough, Manatee). In one flock of eight located near the center of horse cases in Hillsborough County, two chickens converted during May and June from no antibody to EE titers of 1:2560 and WE, 1:80. Of the mammalian sera examined, three have shown low titers to SLE (HAI) and one was positive for California (HAI) antibodies.

Through the courtesy of Dr. Donald McLean of Toronto, Canada, an additional serologic test for Powassan antibodies was employed for selected mammalian sera collected in the fall of 1963 and early 1964. Of the 200 cotton rats, cotton mice, and raccoons that were tested, seven had HAI titers to Powassan antigen ranging from 1:10-1:40. Five were subjected to neutralization test, and one was considered positive. With this suggestive evidence of tick-borne virus activity, 35 pools of ticks were collected from rabbits, raccoons, and opossums trapped for serologic studies. In three viral agents have been obtained; one from the tick Haemaphysalis leporis-palustris collected from a rabbit, one from Dermacentor variabilis collected from an opossum, and one from the same species collected from a raccoon. Identification of these agents is in process.

In addition to these routine surveillance activities at the center, special studies have been concurrently carried out during 1964.

A measure of inapparent human infection with SLE, Tensaw, and California viruses was obtained by rebleeding 366 individuals who participated in serologic surveys in early 1963 in Clearwater (Pinellas County), Tampa (Hillsborough County), Bradenton and Palmetto (Manatee County). Two from Clearwater and one from Hillsborough developed SLE-HAI antibodies over the twelve month interval; one in rural Hillsborough demonstrated both HAI and SN antibodies to Tensaw virus; there were no apparent infections with California virus. In none of the infected individuals was there an associated clinical illness.

A special follow-up examination clinic for post-encephalitis cases was held in cooperation with the USPHS Accident and Aging Study Center in St. Petersburg, and the Pinellas County Health Department. From March 11 through June 19, 224 persons were examined; 96 of these were recovered encephalitis patients, and 128 were normal individuals, matched by age, race, sex, education, and occupation for comparison with the cases. Tests on these individuals included those for psychomotor and psychologic performance, vision, static, and dynamic balance, the Cornell Index, an accident history and arbovirus serologic studies.

In cooperation with the Sunland Training Center in Orlando and the University of Pittsburgh Graduate School of Public

Health, an exploratory study was made to evaluate the possible relationship of arbovirus infection to severe neurologic damage leading to admission to the Sunland Hospital. Of fifty-six children examined, two had significant antibodies (HAI) to EE, and one had SN antibody to California group virus. These were not considered greater than the expected number in a normal population.

Special Biological and Entomological studies included: 1) Laboratory infection experiments with SLE virus in chickens, doves, sparrows, and rats; 2) experiment to determine whether HAI antibodies are transmitted transovarially in chickens; 3) host-attraction studies for wild-caught mosquitoes; 4) detailed field studies of the biology of Culex and Aedes species in the Tampa Bay area; 5) a continuing investigation of the relationship between oviparity status of Culex spp. and time, location, method of collection, rainfall, temperature, and male population density factors; 6) measurement of breeding bird densities in different ecological sites within Pinellas County; 7) screening of vertebrate tissues through two to four day old chicks for serologic conversion prior to suckling mouse isolation attempts and 8) evaluation of different wild and domestic animal sentinel systems using parakeets, pigeons, blue jays, one-half-day old chicks, guinea pigs, wild cotton rats, and opossums.

REPORT FROM DR. J.B. NICHOLS
DIRECTOR OF VETERINARY PUBLIC HEALTH
FLORIDA STATE BOARD OF HEALTH, JACKSONVILLE, FLORIDA

As a part of the statewide encephalitis surveillance and control program, personnel of the Division of Veterinary Public Health initially selected 38 different counties late in 1963 for the purpose of collecting blood specimens for serologic studies from small backyard chicken flocks. The selection of the counties was made with a view of covering as much of the state as possible and at the same time include the general areas where equine cases had been reported during 1963. Every effort was made to obtain birds of the year, i.e., those which had been hatched since January 1, 1963. Since serologic studies made of chicken sera during the previous year did not reveal much reactivity in the northern third of the peninsula, and the panhandle area, a few birds more than one year of age were bled in that part of the state. Those in the remainder

of the state were birds of the year. A total of 491 chickens from 57 flocks were bled in 38 counties. While an effort was made to obtain 10 birds from each flock, a lesser number were bled in several instances when less than 10 were available. While at least one flock was bled in each of the 38 counties, as many as five flocks were bled in those counties were more activity was suspected due to the reported number of equine cases and other factors.

The hemagglutination inhibition (HAI) tests were performed at the Florida State Board of Health Laboratories, Jacksonville. Antigens for Eastern equine encephalitis (EEE), western equine encephalitis (WEE), and St. Louis encephalitis (SLE) were used in the battery. The following tables illustrate in brief the results of these tests.

A Comparison of Serological Examinations

	EEE	WEE	SLE
Total reactors/total birds	126/491	60/491	21/491
Reactor flocks/total flocks	36/57	15/57	13/57
Counties with reactors/total counties	29/38	13/38	12/38

Reactivity by Regions

Region	No. Counties Tested	No. Chickens Tested	EEE No.	EEE %	WEE No.	WEE %	SLE No.	SLE %
Panhandle	13	158	77	48	48	30	5	3
N. Florida	13	142	26	18	11	8	3	2
C. Florida	8	136	15	11	1	1	7	5
S. Florida	4	55	8	14	0	0	6	11

Titers showing dilutions of 1:10 or greater were considered reactive. The highest titer noted in EEE was 1:320, in WEE 1:1280, and SLE 1:640. Reactivity varied from 0 to 100% in each flock tested.

These serologic studies have revealed the areas of greatest viral activity. The amount of reactivity for SLE was not as great as was noted for EEE. The relatively low rate for SLE might be compared with the absence of human cases in 1963.

The heaviest concentration of both EEE and WEE was noted in the eastern part of the panhandle, and the adjoining upper portion of the gulf coast of the peninsular part of the state. The HAI tests are considered to be meaningful when large numbers of specimens are examined.

In order to concentrate further on the surveillance program for the summer and fall of 1964, chicken flocks were placed in the vicinity of three of the larger metropolitan areas, and in the panhandle portion of the state where the above mentioned heavy activity was noted following the 1963 mosquito season. Three different flocks, each with 15 to 20 sixty-day-old birds, were placed at especially selected sites in the areas mentioned above. These sentinels will be bled every two weeks throughout the mosquito season so that the development of titers may be closely observed. In addition, morbidity and mortality reporting of equine encephalitis is being secured from private practicing veterinarians. Each clinically diagnosed case is reported by telephone to the State Public Health Veterinarian in order that up-to-date information can be supplied to the Bureau of Entomology and others concerned. Unvaccinated horses are considered to be excellent sentinels and the prompt reporting of each case provides essential data which is used in connection with the mosquito trapping program. As of June 30, 1964, a total of 55 equine cases had been reported. While this figure is considerably higher than the number of cases reported for the same period in 1963, the close liaison developed with equine practitioners has resulted in more accurate reporting.

To further enhance the sentinel system, the State Game and Fresh Water Fish Commission agreed to warn all licensed game farm operators of the prevalence of arthropod-borne encephalitis and to encourage them to make use of the facilities of the state poultry disease diagnostic laboratories whenever sudden die-offs occurred in pheasants and chuckars. Serological findings and virus isolations of specimens examined are then promptly reported to the veterinary division of the Florida State Board of Health. An epidemiological survey is performed of each outbreak by a representative of the veterinary division.

REPORT FROM DR. ALEXIS SHELOKOV
LABORATORY OF VIROLOGY AND RICKETTSIOLOGY
DIVISION OF BIOLOGICS STANDARDS
BETHESDA, MARYLAND

In December 1963, Dr. Shelokov, formerly Chief, Laboratory of Tropical Virology, National Institute of Allergy and Infectious Diseases, became Chief, Laboratory of Virology and Rickettsiology, Division of Biologics Standards. A small Arbovirus Unit under his supervision has been now authorized. The mission of the unit is: 1) to establish an arbovirus facility with competency in the techniques of animal work, tissue culture, and serology for the study of arthropod-borne viruses; 2) to establish a reference collection of arboviruses for the immediate and future needs of the Division; and 3) to utilize these resources in research on selected arboviruses, particularly those of importance as vaccine candidates.

Work commenced in January 1964, and it is yet too early to report on scientific matters.

In January, Dr. Shelokov was joined by Dr. Nicola M. Tauraso, formerly with him in the Laboratory of Tropical Virology.

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

This report reviews field and laboratory studies on arboviruses during the period May 1, 1963, through April 30, 1964.

Investigations were continued on the overwintering persistence of arboviruses in Kern County. Prior reports summarized isolations of WEE virus from Zonotrichia leucophrys, Mus musculus, Citellus nelsoni, and Culex tarsalis. In addition, a new arbovirus, Buttonwillow virus, was recovered from Sylvilagus audubonii and Lepus californicus. In current studies, two additional strains of Buttonwillow virus were isolated from S. audubonii and Z. leucophrys. From January collections, two strains of

a virus closely related to Trivittatus virus were isolated from Culiseta inornata, and an adenovirus was isolated from the blood of Peromyscus maniculatus. In midwinter studies, no additional WEE or SLE viruses were isolated from C. tarsalis nor by xenodiagnostic feedings of C. tarsalis on 575 birds and 222 small mammals. The midwinter population of White-crowned Sparrows, that had yielded three virus isolations, is composed almost exclusively of Zonotrichia leucophrys gambelii. This subspecies breeds in Alaska and Western Canada, being only a winter resident in Kern County.

The fourth year of study was completed to evaluate intensive C. tarsalis control as a means of suppressing WEE and SLE virus transmission. The C. tarsalis population was held at a very low level in the controlled area. However, while WEE and SLE viruses were delayed in their appearance, the critical threshold level of vector population was not reached where virus transmission ceased.

A major effort has been made to expand knowledge on those aspects of vector bionomics that are most closely related to the maintenance of arboviruses. A three year quantitative and qualitative study on the host range of C. tarsalis in Kern County indicates that this mosquito: feeds predominantly on passerine birds, particularly in the winter; has a significant increase in feedings on doves and mammals in midsummer; has feeding patterns that reflect the abundance and species composition of host populations in different ecologic habitats; feeds rarely on man, rodents, or cold-blooded hosts. Feeding patterns of C. tarsalis collected in the Sacramento Valley and near Greeley, Colorado were quite similar to those observed in Kern County.

Blood meals from Culex thriambus, Culex peus, Culex quinquefasciatus, Culex pipiens, C. inornata, Anopheles franciscanus, and Anopheles freeborni were collected in the above three areas. The Culex fed mostly on birds; the Anopheles on mammals, particularly rabbits; and the Culiseta almost exclusively on large mammals.

The "Detinova technique" for separating parous and nulliparous female mosquitoes has now been applied to over 17,000 C. tarsalis collected through a two-year period. The results indicate that overwintering females probably are insignificant as a virus reservoir, although repeated feedings by this population in the spring could initiate

transmission cycles if infected hosts were present. Virus can be isolated readily from parous females collected in midsummer.

An intensive study has been made on small mammals as arboviral hosts. A 40-acre grid trapping program has been operated monthly for one year on the desert margin of agricultural land. Over 1,900 animals have been captured, ear-tagged, and released, and these animals have now been captured over 5,700 times on initial and recapture attempts. Detailed records are accumulating on age, sex, reproduction, numerical abundance, home range, and ectoparasites of each species. Each month a portion of the animals are bled and their ectoparasites removed, both for virologic study. Some animals have been re-bled as many as 8 times in an 11-month period. The virologic testing program on these samples is just starting and results are fragmentary at this time.

Data have been summarized from virologic studies of over 2,300 small mammals that were collected before the beginning of the grid study. There is little evidence of WEE virus infection in small mammals other than the two virus isolations in the early spring and an occasional HAI positive plasma. HAI antibodies to Group B viruses are found in many species of small mammals. The specificity of Group B antibodies is still uncertain as they are not confirmed by neutralization tests or virus isolations. HAI responses to Powassan antigen had their highest prevalence in recent years. HAI responses to California and Bunyamwera group antigens had a low frequency. No virus was isolated from a large number of ectoparasites collected from these mammals.

Studies on hemophagous Diptera have been expanded to include Culicoides and Phlebotomus. Two isolations of Buttonwillow virus were made from Culicoides variipennis.

General epidemiologic observations in 1963 indicated very low levels of WEE and SLE virus activity in Kern County. An 8-day-old infant apparently contracted WEE infection transplacentally or at birth from her mother who had a mild or subclinical infection. Two of three suspected horse cases were confirmed as WEE. Serologic conversion rates to WEE and SLE viruses in chicken flocks were almost zero in urban flocks and at a low level in rural flocks. It was an unusually cool summer and the control program held vector populations to small numbers in most areas.

Studies of human and chicken sera have not indicated frequent infection with arboviruses other than WEE and SLE.

The isolation of WEE virus from rodents and birds in mid-winter and early spring led to a study of the pathogenesis of this virus in representative species of these hosts. Dipodomys nitratoides, Dipodomys heermanni, P. maniculatus, C. nelsoni, and S. audubonii were inoculated subcutaneously with 1,000 to 10,000 PFU of three different strains of WEE virus. Both species of Dipodomys had high mortality rates within two to six days. Death or paralysis was rare in the other species. All species circulated sufficient virus for two to four days to infect C. tarsalis. HAI antibodies developed in all surviving animals and persisted for 189 days in all species except P. maniculatus. White-crowned sparrows were highly susceptible to three strains of WEE virus, as 60 to 85 per cent of birds died three to eight days postinoculation. With two additional strains, death was delayed until 6-12 days; and with an attenuated strain, all birds lived. All birds developed very high titered viremias within 24 hours and viremia persisted at high levels for at least 96 hours. All survivors developed high levels of HAI antibodies and antibody persisted but decreased in titer three months postinoculation. When six additional species of birds were inoculated with a highly pathogenic virus, Tricolored Blackbirds, Red-winged Blackbirds, and Golden-crowned Sparrows had high mortality; however, House Finches, English Sparrows, and Brown-headed Cowbirds were quite resistant and had inapparent infections.

An attenuated WEE virus has been developed by Dr. H.N. Johnson as a vaccine candidate. This virus still produces viremia in chicks and is infectious to C. tarsalis. Passage in mosquitoes did not alter the characteristics of this clone.

Culex tarsalis did not become infected after ingestion of serum neutralized WEE virus. It appears that the mosquito cannot dissociate infectious virus from antibody by digestion.

Nonspecific hemagglutination and hemagglutination-inhibition continues to pose a serious problem with certain sera and antigens. An inhibitor of EEE viral hemagglutinin develops in chicken serum coincidentially with their maturation,

particularly in female chickens. Ordinary acetone extraction will not remove this inhibitor.

Several new viruses have been characterized further. Buttonwillow is a member of the California complex with slight crossing to Guaroa. Culex tarsalis and A. franciscanus can be infected by feeding on this virus. The cottontail rabbit has a low level viremia following peripheral inoculation.

A virus isolated from C. inornata is closely related antigenically to Trivittatus virus. A virus isolated from C. tarsalis is closely related to Cache Valley virus (Bunyamwera group). An adenovirus was isolated from the blood of P. maniculatus and a possible second adenovirus from C. nelsoni.

Fluorescent antibody procedures have now been adapted to study the antigenic relationships of Group A and B arboviruses. Preliminary data would indicate that this is a rapid and sensitive procedure for detecting viruses in fluid tissue culture systems. Conjugated antisera are now being used for the rapid detection and identification of viruses isolated from mosquito pools. Dual infections with WEE and SLE virus are readily detected.

Studies have been continued on the problems of producing specific antisera to avian serum antigens by using the phenomenon of immunologic tolerance to minimize heterologous responses. Results are promising but still require further development. The dosage of antigen required to produce tolerance is least when the donor is closely related phylogenetically to the bird that is being converted to a tolerant state.

Virus isolation and serologic systems are being developed for 14 arboviruses that may occur in Kern County.

This report represents the summary of an Annual Project Report. A limited number of copies of the detailed report are available on request.

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

A WE outbreak occurred in the Texas High Plains in 1963. It centered around Hale County. Laboratory confirmation was obtained on 31 cases. A few other doubtful cases were also encountered. No case of SLE was found.

Ten pools of Culex tarsalis mosquitoes collected in this area yielded WE virus. The first pool of mosquitoes found to harbor virus was collected July 30 and the last on August 7. Abundance of C. tarsalis in the High Plains area is mainly associated with irrigation practices, rather than amounts of rainfall. In most areas of the state, subnormal amounts of rainfall were recorded.

Blood samples from sentinel flocks of chickens stationed in several areas of the state were tested for activity against western, eastern, and St. Louis arboviruses. Flocks had not been put out in the general area where human and equine cases were confirmed. Significant WE antibody was found both in north and south Texas and in El Paso, but little or no activity was noted in most areas of east Texas. Not all of the sera have been completely tested. There was little or no evidence of SLE or EE virus activity in the areas tested.

REPORT FROM DR. S.S. KALTER
SOUTHWEST RESEARCH CENTER, SAN ANTONIO, TEXAS

In a previous communication (J. Bacteriology, 87, 744-746, 1964) it was reported that very few of our animals had HI antibodies to the various arboviruses. With the exception of one animal which had a low titer (1:10) for yellow fever, all other animals were negative. This question was of concern to us inasmuch as all animals are vaccinated with yellow fever vaccine prior to shipping to the United States. It was therefore thought advisable to study this problem a little further.

Neutralization tests using the 17D strain of yellow fever virus (kindly supplied by Dr. Telford Work, Communicable Disease Center) were used to test pre- and post-vaccination serums obtained on animals prior to shipment to this laboratory from our facilities in Kenya, East Africa. Post

vaccination specimens were obtained at least 14 days folling vaccination. The test was done in young mice approximately 3-5 weeks of age, 5 mice per dilution, using 10-fold serial dilutions of virus with an equal volume of serum. The results may be seen in the following table. It will be noted that one log or better protection was obtained only in half the animals. This study is being continued attempting to explain the differences observed.

LD_{50} Titer (Neg. Log_{10}/0.03 ml)

Animal #	Pre Vac.	Post Vac.	Animal #	Pre Vac.	Post Vac.
A-995	4.5	<3.0	A-1	4.8	2.5
A-994	4.0	3.7	A-2	>4.0	4.3
A-990	4.5	3.4	A-3	4.5	3.6
A-998	4.5	3.5	A-4	4.0	2.8
A-991	3.5	3.4	A-5	3.7	3.3

Stock Virus Titer	Neg. Cont. Serum	Pos. Cont. Serum
5.0	>2.0	<3.0
4.8	>2.0	<3.0
4.6	>2.0	<3.0

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

The studies on experimental arbovirus infection in bats which are in progress in this laboratory have been designed to define the various facets of the infectious process in these animals which might enable them to function as effective reservoirs for these agents in nature. Recent experiments have been concerned with the characteristics of infections in animals maintained at 24°C versus infections in animals maintained at low temperature (5-10°C). The rationale behind such experiments is the phenomenon of hibernation common to many bat species. The thermoregulatory mechanism of these animals is such that the body temperature of the resting bat equilibrates with the temperature of the environment over a range of 2 to 37°C. In nature, therefore, hibernating bats in temperate zones experience long periods of low body temperature lowered, but metabolic rates of these animals are reduced significantly. Studies on the influence of low temperature

on experimental arbovirus infections in bats seemed indicated, therefore, in order to determine if these animals might sustain infection throughout periods of hibernation and thereby serve to overwinter these agents in temperate zones.

Three species of insectivorous bats were used in these studies, the Mexican free-tailed bat (Tadarida b. mexicana), the little brown bat (Myotis l. lucifugus), and the big brown bat (Eptesicus f. fuscus). Two strains of Japanese B encephalitis (JBE) virus and one strain of St. Louis encephalitis (SLE) virus were employed and the virus dose consisted of 150 mouse intracerebral LD_{50} ($MICLD_{50}$) administered subcutaneously. Experiments were planned so that periods in which animals were held at low temperature in the laboratory corresponded to the season of natural hibernation in an effort to induce true hibernation in the bats rather than attain merely a state of hypothermia. Groups were placed in the cold rooms at varying times following virus inoculation to represent (1) animals entering hibernation immediately after becoming infected and before infection develops to a demonstrable level, (2) the uninfected bat which acquires infection during the period of hibernation from an infected mosquito seeking a blood meal, and (3) animals entering hibernation at the peak of the infectious cycle when virus is present in blood and other tissues. During periods at low temperature, groups of bats were tested for viremia alone or were sacrificed and brown fat, brain, and kidney, as well as blood, were assayed to determine sites of virus sequestration. After varying times at low temperature, animals were transferred back to room temperature and tested for arbovirus infection.

An analysis of data accumulated on the influence of environmental temperature on experimental arbovirus infection in bats which is now being assembled for publication indicates that these animals would be ideal hosts for overwintering these viral agents. The results obtained allow us to speculate along several lines. 1. Bats which enter hibernation with high titers of virus in blood and tissues apparently suffer a prolonged infection, possibly due to the suppression of antibody production in the cold. These animals could provide infective blood for at least a month for mosquitoes present at the hibernating site. In addition, the bat which enters hibernation in a viremic state, although his blood titer gradually decreases during the

winter, would be capable of circulating virus again upon arousal in the spring. In this regard, it is of interest to note that virus can be demonstrated frequently in the interscapular brown fat of the cold-exposed, non-viremic bat, suggesting that this tissue sustains virus particles during periods of suppressed metabolic activity. Only occasionally was virus demonstrable in brain and kidney tissue of the hibernating bat. 2. Bats which enter hibernation soon after receiving an infective dose of virus, but before infection has developed to demonstrable levels may, in fact, support cycles of viral multiplication even after body temperatures are lowered and metabolic rates decreased. The period of transition from the active to the hibernating state apparently provides sufficient biological activity for virus replication. In certain bats, blood virus titers could reach levels sufficiently high to provide infective blood for mosquitoes present at the site of hibernation. In addition, these animals would be capable of sustaining infection through months of winter hibernation and upon arousal in the spring suffer a more intense viremia. 3. The bat in a state of deep hibernation could receive an infective dose of virus from a mosquito present at the site and although bats in such a depressed metabolic state would not support cycles of virus multiplication sufficient to produce demonstrable viremia, the infection would be sustained throughout winter sleep and upon arousal in the spring an intense viremia would occur.

These studies demonstrating experimentally the various conditions under which the hibernating bat can serve to overwinter an arbovirus add strong supportive evidence to the growing concept of the role of hibernating animals in the maintenance of these agents in nature during periods when vectors are not active.

REPORT FROM THE DISEASE ECOLOGY SECTION USPHS, COMMUNICABLE DISEASE CENTER GREELEY, COLORADO

I. Report from Greeley, Colorado, Dr. A.D. Hess, Dr. L.C. LaMotte, Dr. Preston Holden, Dr. George W. Sciple, and Dr. Richard O. Hayes.

Dr. Richard O. Hayes has recently been transferred from the Taunton, Massachusetts, Field Station to the Disease Ecology Section, Greeley, Colorado. Dr. Hayes assumed his duties as Chief of the Biology and Control Investigations in June, 1964.

Host Preference and Seasonal Feeding Habits of Mosquitoes

The micro-precipitin technique developed by Tempelis et al., at the School of Public Health, University of California, has made it possible to determine the animals upon which engorged mosquitoes have fed. Mosquitoes collected in Colorado and tested by Dr. Tempelis have shown marked seasonal changes in feeding habits. Culiseta inornata fed almost exclusively on mammals throughout the season; Culex tarsalis, however, fed mostly on birds in the spring but gradually increased its feeding upon mammals during the summer, reaching a peak of 80 per cent in September. Culex pipiens was found to feed almost exclusively upon birds during the July-August period, even though large numbers of cattle and horses were readily available in the areas. Limited numbers of C. tarsalis and C. quinquefasciatus collected in Hale County, Texas, had similar host preferences to the C. tarsalis-C. pipiens in Colorado. Availability may determine the species of bird from which the bird-feeding mosquitoes obtain a blood meal, but this does not seem to be an important determinant in the selection of mammals versus birds.

Colorado Tick Fever

A study of the ecology of CTF virus in an endemic area of Colorado was initiated recently. The cooperation of 103 Boy Scouts and their leaders from Wichita, Kansas, and Midland, Texas, was enlisted with the assistance of the local BSA camp officials. These Scout troops were scheduled to spend a week at the BSA camp in a mountain area of Colorado where CTF has been active in recent years.

During the Scouts' stay, tick transects were run in the area adjacent to the campsites. A total of 274 Dermacentor andersoni was collected on 15-17 June on five 1000-foot transects in tall grass and densely covered areas; ticks were not detected in open short grass areas or in those areas with an overstory of pines. Attached ticks were reported, removed, and recorded by the staff. Home-town physicians have been asked to cooperate by notifying us of any CTF-like disease in the boys after their return. Post-exposure sera have been collected from about half of the boys. Ticks became scarce in this area by early July; only three ticks were collected from those transects which were most productive during mid-June.

Viruses Isolated from 1963 Texas Mosquitoes

As indicated in the last Information Exchange, WE virus was responsible for approximately half of the human encephalitis cases during the outbreak in the Texas Panhandle in 1963. WE virus was isolated from 69 mosquito pools, and SLE from 5 pools. Thirty-one isolations were made of a virus serologically related to Hart Park virus and Turlock virus was isolated from 5 pools. Since half of the human cases of encephalitis failed to show a rise in antibody to either WE or SLE, sera from 47 patients were tested against one of the Hart Park isolates. None of these sera had demonstrable CF titers. Seven convalescent sera from selected patients also failed to show neutralizing antibody against Turlock.

REPORT FROM DR. CARLOS CAMPILLO-SAINZ,
QBP. RAUL RUBIO-BRITO, AND DR. JULIO DE MUCHA-MACIAS,
INSTITUTO NACIONAL DE VIROLOGIA DE LA S.S.A.
MEXICO D.F., MEXICO

The present preliminary report deals with the serological surveys in birds. This study has been undertaken in the Instituto Nacional de Virologia de la S.S.A. as part of its research program on arboviruses.

All the studied plasma are from birds captured and identified by Dr. Robert Dickerman. They were captured, either shot or trapped, in Coatetelco (State of Morelos), Tlacotalpan (State of Veracruz) and San Blas (State of Nayarit). See Figure No. 1.

Blood was taken by jugular or cardiac puncture with a syringe with heparin (1 mu in 0.1 ml saline per ml of blood).

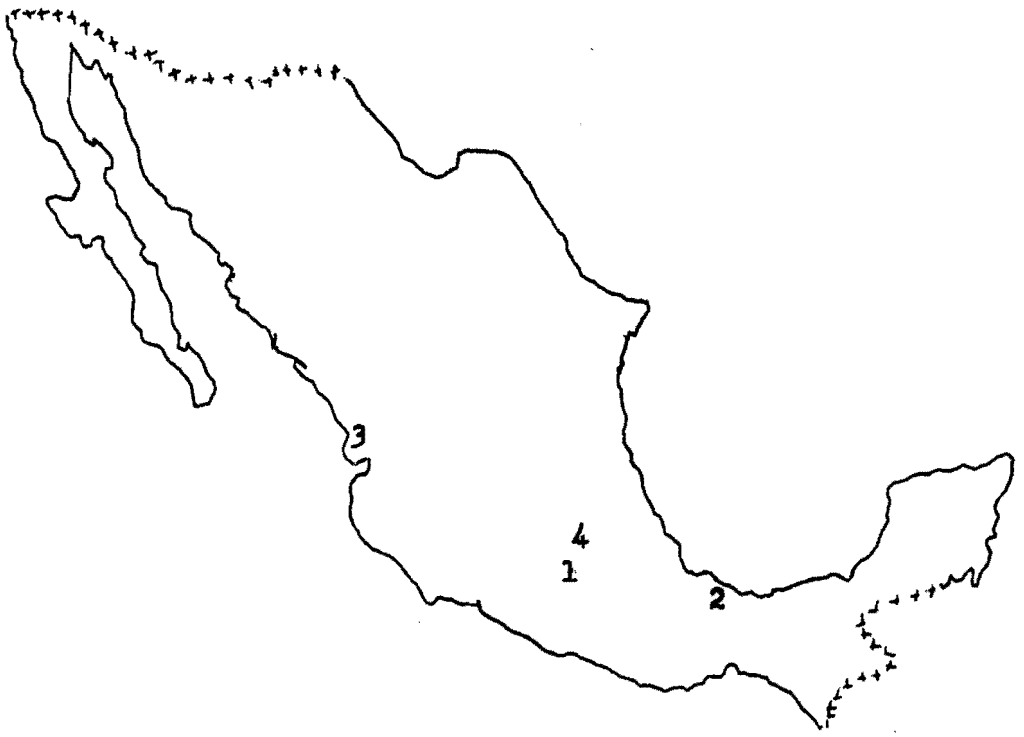
The presence of antibodies was determined by the plaque neutralization test using the chick embryo cell microculture technique in leucite trays.

The neutralization index of each serum was calculated against SLE virus and WEE virus or both. Sera with a neutralization index of 1.7 log. or more were taken as positives.

The accompanying table shows the pertinent data on the birds found positive among the 68 which were tested.

F I G U R E No. 1.

AREAS IN WHICH BIRDS WERE CAPTURED.



1.- COATETELCO, MORELOS..
2.- TLACOTALPAN, VERACRUZ.
3.- SAN BLAS, NAYARIT.
4.- DISTRITO FEDERAL (I.N.V.)

INSTITUTO NACIONAL DE VIROLOGIA DE LA S.S.A.

SEROLOGIC SURVEY IN BIRDS FROM MEXICO.

SPECIMEN*	NUMBER	DATE OF CAPTURE.	NT RESULTS SLE	NT RESULTS WEE
American Egret (Casmerodius albus egretta)	5	Sept/22/62	3/5**	1/5
Little Blue Heron (Florida coerulea coerulea)	8	Sept/21-22/62	5/6	1/6
Lousiana Heron (Hydranassa tricolor ruficollis)	6	Sept/21-22/62	0/5	1/6
Boat-billed Heron (Cochlearius cochlearius zeledoni)	3	Sept/22/62	1/2	0/3
Green Heron (Boturides virescens)	2	Sept/21/62	1/2	0/2
Total.....	24		10/20	3/22

* Nestling birds captured in San Blas, Nayarit.

** Positives/ Studied.

REPORT FROM DR. JOSE SOSA-MARTINEZ*,
CHIEF, INFECTIOUS DISEASE RESEARCH LABORATORY,
HOSPITAL INFANTIL DE MEXICO, MEXICO 7, D.F.
AND
DR. FRANCISCO BIAGI F.**,
HEAD, DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY,
SCHOOL OF MEDICINE
UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, MEXICO 21, D.F.

During the month of January, 1964, both institutions combined forces in order to make a virological and entomological study in a central jungle area of the Yucatan Peninsula, corresponding to the territory of Quintana Roo.

Blood was obtained from adult humans and also from some of the animals peculiar to these regions. A high percentage of these blood samples showed neutralizing antibodies against St. Louis encephalitis and Ilheus viruses.

A total of 4,138 mosquitoes was collected using human bait in two expeditions, and after being kept alive for 24 hours, they were frozen, packed in dry ice, and then flown to Mexico City.

The suspensions of the mosquitoes are being inoculated into suckling mice by the intracerebral route, as well as in tissue cultures from chick embryos and in hen embryonated eggs.

The following is the list of female mosquitoes collected, according to species and number:

Mansonia (Rhynchotaenia) nigricans (Coquillet, 1904)	127
Mansonia (Mansonia) indubitans Dyer & Shannon, 1925	182
Psorophora (Janthinosoma) ferox (Humboldt, 1820)	47
Aedes (Ochlerotatus taeniorhynchus (Wiedemann,1821)	715
Aedes (Ochlerotatus serratus (Theobald, 1901)	2268
Aedes (Ochlerotatus) spp.	603
Aedes (Finlaya) terrens (Walker, 1856)	5
Aedes (Howardina) spp.	185
Sabethes (Sabethoides) chloropterus (Humboldt, 1820)	6

*Supported by a grant from The Rockefeller Foundation.
**Supported by Grant No. AI 210 from the National Institutes of Health.

REPORT FROM THE MICROBIOLOGY DEPARTMENT UNIVERSITY OF THE WEST INDIES, JAMAICA

An epidemic of dengue-like fever which started in Jamaica during the second quarter of 1963 and persisted until early 1964 is the main project that is being investigated by the Department of Microbiology, University of the West Indies.

The total dengue-like cases notified was approximately 1,500. About 950 specimens were investigated serologically. Sixty-five primary inoculations were made into tissue culture and 353 inoculations into suckling mice in attempts to isolate the causative virus.

Serological results gave a 36.5% positivity of 4-fold or greater conversions against dengue types 1, 2, 3, and 4. No definite isolation of the virus has been made, but from the results of tissue culture inoculations, mice inoculations, and fluorescent microscopy, we are convinced of having about eight potential isolations.

To date, no virus isolation has been made from birds and rodent specimens. However, about ten birds gave antibodies to EEE. These birds were mostly warblers, North American migrants. Only one rodent gave antibodies to EEE virus.

A bird-banding program has been established since April, 1964. The essence of this is to collect paired sera from these birds to detect antibodies to EEE virus and ultimately to determine if this virus is introduced into the island by migrant birds.

Dr. C. Brooke Worth of the Trinidad Regional Virus Laboratory has initiated this bird-banding program which is operative in the parish of St. Thomas (the area of the EEE epidemic of 1962) and in Portland, a neighboring parish.

REPORT FROM SUNTHORN SRIHONGSE, PEDRO GALINDO, AND MARGARET GRAYSON, GORGAS MEMORIAL LABORATORY, PANAMA

Prevalence of Group C arboviruses in Almirante, Panama:

Seven distinct antigenic entities belonging to the group C of arboviruses have been reported from South America to date (Shope and Causey, 1962). Rodaniche et al., (in press)

have added two new types of this group from Panama, Madrid and Ossa viruses, isolated from mosquito collectors working in the Almirante area.

During the past five years, over 200 strains of arboviruses have been isolated from Almirante, a tropical rainforest area on the Atlantic coast of Panama (reported, in part, in Infoexchange #5). Sixteen out of 50 isolates obtained from rodents during 1961 and 1962 turned out to be members of Group C distributed into 5 antigenically distinct types. Ten of these agents were yielded by sentinel mice, five were obtained from the Chiriqui Cotton Rat (Sigmodon hispidus chiriquensis) and one from Panama's Spiny Rat (Proechimys semispinosus panamensis). This report presents data on the primary isolation of the 16 strains, as well as preliminary results of serologic investigations aimed at their identification.

Antigens prepared from the sixteen strains were first screened by CF tests against mouse hyperimmune sera of the following antigenic types of arboviruses: VEE, EEE, WEE, Pixuna, Mayaro, Aura, Una, Guama, Guaroa, Wyeomyia, Kairi, Cache Valley, California, Melao, Oropouche, Capim, and Turlock. No fixation of complement was observed in these screening tests. However, positive results were obtained with some of the seven group C type-strain antisera also used in these preliminary tests. Attempts were made to prepare HA antigens from infected suckling mouse serum for all of the isolates under study, according to the technique described by Shope and Whitman (personal communication). Although difficulties have been frequently experienced, many workable HA antigens have been obtained, thus making possible the running of preliminary tests toward characterization of these isolates. Immune serum for each isolate was prepared in adult mice by one-injection inoculations of live virus prepared from infected suckling mouse livers. For preparation of antisera of Group C type strains, the same procedure was used, except that one injection of formalinized virus preceded the live virus injection. In Apeu, formalinization of virus in the first injection was omitted as unnecessary.

A number of cross HI tests were run with the unidentified isolates and eight of the nine known type-strains of group C viruses with the following results. Four isolates from sentinel mice (BTSM 122B, 147A, 411A, and 435A) were found to be closely related to Caraparu virus (see Table I).

Three strains from sentinel mice (BTSM 116B, 127A, and 127B) showed close antigenic relationship with Madrid virus (see Table II), and one agent obtained from Panama's Spiny Rat (BT 4968) reacted with Nepuyo virus (see Table III). The remaining eight strains were found to differ from the 7 original types of Group C viruses from South America as well as from Madrid virus. Material of Ossa virus, originally isolated from Almirante, could not be obtained in time to include it in these preliminary tests. The eight unidentified strains separate into 2 distinct antigenic types (see Table IV). The first type consists of 2 isolates from the Cotton Rat (BT 4971 and 4972) and one isolate from sentinel mice (BTSM 117A). The second type is represented by 3 isolates from the Cotton Rat (BT 5012, 5015, and 4975) and 2 isolates from sentinel mice (BTSM 109A and 389A). While these two types are separable by HI tests, CF grid titrations demonstrate close relationship between them.

HI relationships between prototypes of the five distinct types of Group C isolates obtained at Almirante and 8 of the 9 type strains of the known Group C agents may be seen in Table III.

Data presented indicate prevalence of Group C arboviruses in the rodent population of Almirante. Five distinct antigenic types of this group could be recognized in HI tests with 16 viral isolates obtained from two species of wild rodents and sentinel mice exposed in the field during 1961 and 1962. The first antigenic type (prototype 147A) shows close antigenic relationships by HI with Caraparu virus. The second type (prototype BT 116B) was found to be identical in HI tests to Madrid virus, which was isolated in the same area from the blood of a mosquito collector. The third type (prototype BT 4968) appeared to have definite and close relationships with Nepuyo virus.

The fourth and fifth types, represented by prototypes BT 4971 and BT 5012, which reacted in low titers with Polyvalent Group C immune serum, could possibly belong to another undescribed group of arboviruses related to Group C. Further tests are required before definite conclusions can be reached.

We wish to acknowledge our appreciation to the Rockefeller Foundation Virus Laboratories in New York and Belem which furnished most of the type strains of Group C agents used in these studies. Investigations herein reported were supported in part by National Institutes of Health Research Grant No. AI-02984.

References:

Shope, R.E., and Causey, O.R. (1962): Further studies on the serological relationships of Group C arthropod-borne viruses and the application of these relationships to rapid identification of types. American Journal of Tropical Medicine and Hygiene, 11:283-290.

Rodaniche, E., Andrade, A.P., and Galindo, P. (1964): Isolation of two antigenically distinct arthropod-borne viruses of Group C in Panama. American Journal of Tropical Medicine and Hygiene, in press.

TABLE I

Results of HI tests of Caraparú virus and 4 new isolates from Almirante

Antisera \ Antigens	Caraparú	BTSM 122B	BTSM 147A	BTSM 411A	BTSM 435A
Caraparú	640	160	>160	320	320
BTSM 122B	160	160	160	---	---
BTSM 147A	160	---	320	---	---
BTSM 411A	320	---	---	320	---
BTSM 435A	320	---	---	---	640

TABLE II

Results of HI tests of Madrid virus and 3 new isolates from Almirante

Antisera \ Antigens	Madrid	BTSM 116B	BTSM 127A	BTSM 127B
Madrid	1280	1280	---	---
BTSM 116B	>320	1280	>320	---
BTSM 127A	1280	>1280	>320	---
BTSM 127B	>1280	>1280	>320	---

TABLE III

Results of HI tests of Group C type strains and 5 distinct prototypes of new isolates

Antisera \ Antigens	Oriboca	Marituba	Murutucu	Itaqui	Apeu	Caraparú	Nepuyo	Madrid	BTSM 116B	BTSM 147A	BT 4971	BT 5012
Oriboca	320	0	0	20	0	0	0	0	0	---	10	0
Marituba	0	160	80	0	20	0	40	20	20	---	0	0
Murutucu	0	80	640	0	40	40	40	40	40	---	20	0
Itaqui	0	0	0	80	0	0	0	0	0	---	0	0
Apeu	0	0	0	0	640	80	0	40	40	20	0	0
Caraparú	0	80	40	0	320	640	40	160	80	>320	0	0
Nepuyo	0	40	40	0	40	40	640	20	20	---	0	0
Madrid	0	20	20	0	---	40	40	1280	1280	---	0	0
BTSM 116B	0	40	20	0	80	80	20	1280	1280	---	0	0
BTSM 147A	0	40	0	0	160	160	0	80	----	320	----	---
BT 4971	40	80	0	20	40	20	0	----	----	---	2560	---
BT 5012	0	0	0	0	---	0	0	0	----	---	20	640
BT 4968*	10	20	0	0	0	40	640	0	0	---	0	0
Poly C	40	320	160	320	640	640	160	20			40	20

*Sufficient quantities of antigen of prototype BT 4968 could not be prepared to include it in this series of tests.

TABLE IV

Grouping by HI tests of 8 new isolates

Antisera \ Antigens	BT 4971	BT 4972	BTSM 117A	BT 5012	BT 5015	BT 4975	BTSM 109A	BTSM 389A
BT 4971	2560	2560	2560	----	----	----	----	----
BT 4972	----	----	----	----	----	----	----	----
BTSM 117A	320	320	640	----	20	40	----	----
BT 5012	----	----	----	640	640	----	----	----
BT 5015	0	----	0	320	640	>80	320	----
BT 4975	20	0	20	640	640	>160	----	----
BTSM 109A	----	10	20	----	320	>320	----	----
BTSM 389A	0	0	0	320	160	>320	>80	160
Polyvalent Group C	20	----	40	20	20	< 20	----	

REPORT FROM MIDDLE AMERICA RESEARCH UNIT (NIAID), PANAMA
(DRS. K.M. JOHNSON, R.B. MACKENZIE, M.L. KUNS, P.A. WEBB)

This report of current activities in the study of hemorrhagic fever in Bolivia is dedicated to the memory of the late director of MARU, Dr. Henry K. Beye, who died suddenly on April 8 of a heart attack. His many friends around the world will be pleased to know that the government of Bolivia formally dedicated the hospital in San Joaquin in his honor on July 25th, and at that time posthumously awarded him the Order of the Condor, the highest formal honor accorded by that country to non-Bolivian citizens.

Hemorrhagic Fever in Bolivia:

Field Studies: After an apparent brief pause in December-January, human BHF illnesses resumed the pattern displayed during 1963 in San Joaquin, with upwards of 25 cases hospitalized monthly in February, March, and April. Continuous surveillance of hospital morbidity, and regular census taking and household surveys for acute morbidity, were instituted. In February and March, a survey of bats was made by Mr. Ed Tyson of Florida State University. More than 20 species were taken in and about San Joaquin. Distribution of bats did not appear to be correlated with the disease. Between March and late June extensive collections of ectoparasites from rodent nests and of mosquitoes were made for virus isolation attempts. The mosquito collections were made by Dr. Paul Woke, Laboratory of Tropical Virology, NIAID, Bethesda, who also carried out experimental transmission studies with four species of culicines.

On May 1, an experimental control program designed to study the effect on human disease of systematic killing of rodents, particularly Calomys calosus, was begun in San Joaquin. This campaign, in which both traps and zinc phosphide were employed, was directed by Maj. S.J. McConnell and Capt. Jack Riddell, U.S. Army V.C. A progressive decrease in hospital admissions among residents of the "treated" portion of the town was observed during the following six weeks. The effect appeared about 10-14 days after initiation of control measures. Rodents in the remainder of the town were attacked on June 15. Again a decline in human disease was noted beginning about two weeks thereafter. There have been no suspect HF cases

admitted to the hospital since July 2 (as of July 23). Since 95% of the rodents killed in this campaign were Calomys callosus, the results appear to provide further support for the concept that this vertebrate may be important in maintaining the disease at epidemic levels among the human population of San Joaquin. Details of this study will be presented in future issues of the Exchange.

Ecological studies were continued during February and March, and again during late May and June to provide a total of 32 weeks in northeastern Bolivia. A light aircraft (type U-10) was made available for a period of six weeks by the U.S. Air Force. This aircraft was used for both low and high altitude photographic coverage of the epidemic centers and to provide daily support for rodent trapping operations in a total of 13 localities in the Department of Beni. The occurrence of seven selected species of rats and mice in these and other localities studied to date are shown in the accompanying table (Table 1).

Virus Studies: The seven isolates reported in the previous Exchange have been further characterized. No significant strain differences were found among five human and two Calomys isolates in reciprocal CF tests. Neutralization studies are incomplete but indicate that all strains are closely related and separable from both Junin and Tacaribe viruses. The agent of BHF has been named Machupo virus after the tributary of the Itenez River that passes about one mile from the town of San Joaquin. A registration card has been prepared for the catalogue, and lyophilized specimens of the Carvallo strain have been deposited with the American Type Culture Collection.

The Carvallo strain has inferentially been found to contain RNA (Table 1). Results of thermal inactivation studies are shown in Table 2. Infectivity was destroyed at $<$ pH 5.0 after two hours of incubation at room temperature, but was well preserved between pH 6.0 and 9.0. Preliminary results suggest that infectivity is fairly stable at -70C. for at least three months, even in the absence of added protein. Attempts to estimate virus particle size have not been technically satisfactory. One experiment using

gradocol membranes resulted in an estimate of about 150-180 mμ. This, perhaps, represents an upper limit; it seems likely that the agent will prove to be somewhat smaller. Complement fixing activity in crude alkaline extracts of infected infant hamster brains has been found to be stable for more than three months at 4°C.

Hamsters five to six weeks of age are readily infected either IC or IP. Illness or death is rarely observed. Titration of infection by measuring CF antibody response indicates that the infectious dose for adult animals is quite close to the lethal dose for infants. Adult hamsters have been found to excrete the virus in urine for at least 120 days. All samples (pooled urine from three animals) tested were positive, beginning eight days after inoculation. These animals have also been shown to possess both CF and neutralizing Machupo virus antibodies. This potentially significant observation is now under study in laboratory-raised Calomys callosus. Laboratory rodent-to-rodent transmission has been noted. High titered CF antibodies were found in sera of 14 of 15 mother hamsters bled about three weeks after ingestion of baby hamsters inoculated with virus at least five days previously. Although mode of transmission cannot be clearly inferred from these results, it seems possible that infection may have been via the gastrointestinal tract. Further studies are in progress.

Table 1

Distribution of Rodents - Department of Beni, Bolivia

Localities	Calomys	Mus	Orysomys bicolor	Orysomys subflavus	Proechimys	Rattus	Zygodontomys
Orobayaya	+		+		+		
San Joaquin	+	+	+	+	+		+
Providencia	+		+		+		
Barranquita	+		+		+		+
San Marcus					+		+
Filadelfia			+		+		
Veintidos							
San Ramon	+			+		+	
Las Peñas			+				+
Azunta					+		
Pto Silas							
Km 5					+	+	
Santiago	+						
Exaltacion							+
Santa Ana							
Santa Rosa		+					
San Juan	+						
San Pedro							
Magdalena	+	+	+	+			
Huacaraje	+						+
Baures	+	+	+				
Bella Vista					+	+	
Lago Victoria		+	+		+		+
El Carmen		+			+		
Ascencion							

Table 2

Effect of Bromo DeoxiUridine on Growth of Machupo Virus in Tissue Culture

Virus	Log_{10} Titer (TCD_{50}/ml.)	
	BUDR(10^{-5}M)	No BUDR
Machupo	6.2	5.7
Vaccinia	< 2.2	6.2
ECHO-11	9.2	8.7

Table 3

Thermal Inactivation

Virus pool in borate saline pH8, no serum.
Initial titer about $10^{5.5}$ $TCID_{50}$/0.2 ml.

Titer After Indicated Incubation Interval

	Minutes			Hours					Days						
Temp	5	15	30	1	6	12	24	36	2	3	4	5	6	10	14
56C	3.5	2.25	<0.7	<0.7											
37C		5.5	5.5	>5.5	>4.5	3.5	3.5	0.75							
24C				6.0	5.25		5.0		3.0		0.7	3.0	<0.7	3.25	2.0
4C						5.75	5.5			3.75					

Infectivity was rapidly lost at 56C. Of some interest was the relative resistance to rapid inactivation at 37C. This finding should permit wider choice of conditions for studying the virus neutralization reaction.

REPORT FROM LABORATORY OF TROPICAL VIROLOGY
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
BETHESDA, MARYLAND

The major interest of this laboratory has remained centered about studies related to hemorrhagic fevers, especially that occurring in Bolivia. Preliminary studies of Junin virus suggested the suckling hamster as a more suitable laboratory animal than the suckling mouse. Table I is a summary of comparative studies of Junin virus infection in suckling mice and hamsters. Although quantitative differences were observed, the hamster has also proven useful in studies of both Tacaribe and Machupo (Bolivian hemorrhagic fever) viruses. The most conspicuous advantages of the suckling hamster are susceptibility to infection by peripheral routes, more sensitive LD_{50} and ID_{50} virus assays, slightly wider range of age susceptibility and slightly shorter incubation periods. Although not shown by the tabulation, repetitive assays in suckling hamsters have been more reproducible than those in mice. The latter characteristic has also been noted by Dr. Johnson at MARU, Canal Zone.

Following the experience at MARU with Machupo virus pathogenicity in suckling hamsters, an in vivo protection test was conducted according to the following scheme, with Tacaribe virus as the initial model. Since adult animals do not usually die after challenge with Tacaribe, Junin, or Machupo viruses, it was necessary to use passive immunity conferred on 4-day-old suckling hamsters by "immunized" mothers. Thus, 14-day-old females were inoculated with live virus and examined for the presence of complement fixing (CF) antibody in their sera. Antibody (1:32 serum titer) was demonstrable by 30 days of age and these immune females were bred to normal males at about 45 days of age. Prior to breeding, a small sample of this female population was sacrificed and assayed for live virus, with negative results. Nonimmune females were similarly treated for the production of normal control litters. The resulting litters were challenged with Tacaribe, Junin, or Machupo virus as shown in Table II. A second experiment has been completed in which passively immune litters were challenged with Tacaribe and Junin viruses, with comparable results. Our interpretation of this data is that passive immunity protected against Tacaribe, the homologous virus, and provided an in vivo model for a measure of protection.

On the other hand, there was no protection against relatively small Junin or Machupo virus challenges. This may be contrasted with the previously described evidence of slight cross neutralization of Junin virus plaques by hyperimmune Tacaribe antiserum.

In similar studies, 14-day-old female hamsters were initially inoculated with Machupo virus, but the majority of these animals died. When this procedure was repeated with 21-day-old females, most survived and demonstrated antibody but very few of them were successfully bred (5 of 30). Those litters have been challenged with homologous virus but results are not available.

Fourteen-day-old females inoculated with chloroform-inactivated virus responded in two different ways. A few demonstrated signs of illness, excreted virus in their urine, and developed CF antibody. It is our interpretation that these animals received live virus that was not inactivated by the chloroform treatment, especially since a small number of plaques were observed after inoculation of MA-111 cell cultures with aliquots of the "inactivated antigen". In contrast, the majority of the "immunized" females showed no illness and failed to demonstrate CF antibody in their sera. These animals were bred without difficulty and the resulting litters challenged with Machupo virus. As shown in Table III, protection was not demonstrable, even after a small challenge of live virus. Thus, while chloroform inactivated virus has consistently demonstrated in vitro antigenicity in the CF test, we must conclude that chloroform treated antigen was not protective in the in vivo model used. Additional studies of the immunizing potential of repeated doses of inactivated virus are in progress, especially by peripheral routes. Incidentally, we question whether or not our experience with female hamsters "immunized" with live virus represents an adverse influence upon fertility. Similar experience and questions have been related by investigators at MARU and further studies are urgently indicated.

Immune ascitic fluids have been recovered from both mice and hamsters after a schedule of weekly intraperitoneal injections of Machupo virus and complete adjuvant, as shown in Figure 2. Ascitic fluid was produced in 12-14 gram female mice without difficulty, but production in female hamsters required selection of older animals - about 3 months of age.

Complement fixing antibody in the first samples of mouse ascitic fluid were lower (1:32-1:16) than in similar samples of hamster ascitic fluid (1:64-1:128). However, in both cases CF antibody levels became progressively lower and the booster effect of re-inoculation on antibody titer was conspicuously absent. Furthermore, volume of ascitic fluid production was reduced after booster inoculations. Hamsters dried out completely and seemed seriously debilitated when they were sacrificed by exsanguination. Continued studies on immune ascitic fluid production are in progress.

Plaque formation and homologous antiserum neutralization by Tacaribe, Junin, and Machupo viruses were reported in the Arthropod-borne Virus Information Exchange No. 9. More detailed studies of virus infection in the continuous rabbit embryo kidney cell cultures (MA-111) have been completed in collaboration with Mr. Monroe Vincent, Microbiological Associates, Inc. Replicate PFU assays (plaque forming units) indicate the ratio of PFU to suckling hamster ID_{50} is less than 1/10 per unit volume. Within the range of 1 to 50 plaques per 2 ounce bottle, the plaque counts have been related to 2-fold virus dilutions. The results of growth curve experiments, measured by PFU assay, are summarized in Figure 1. All TC harvests were assayed for CF and hemagglutinating antigen with negative results. Reproducible CPE was not observed in tube or bottle cultures in fluid medium. Continuing studies are directed to defining the dynamics of serum neutralization, maximum virus production and the possibility of producing and recognizing virus attenuation through serial passage and plaque characteristics.

We are pleased to announce the addition of Dr. Bunsiti Simizu to our staff as a Visiting Associate. Dr. Simizu brought a continuous line of monkey kidney TC (designated VERO) that may prove similar to the BSC-1 line of green monkey kidney. Preliminary studies have demonstrated plaque production under agar and CPE under fluid by Tacaribe virus, and $TCCPD_{50}$ titrations seem equivalent to PFU assays. Studies in progress include plaque production and CPE by Junin and Machupo viruses.

Table I. JUNIN VIRUS: COMPARISON LD_{50} AND ID_{50} ASSAYS ($1/log_{10}$) IN MICE AND HAMSTERS

Animal Age	MOUSE LD_{50}			HAMSTER ASSAY								
				0.02/ic Route			.03/ip Route			.02/sq Route		
	.03/ip	.02/ic	ST*	LD_{50}	ID_{50}	ST	LD_{50}	ID_{50}	ST	LD_{50}	ID_{50}	ST
1 day	<2	5.9	10-20	6.3	7.5	8-16	4.2	6.3	13-16	1.9	5.3	11-24
4 day		6.1	7-20	5.0	6.6	8-16	5.2	≥6.1	13-18	1.4	5.2	15-28
10 day		5.1	10-16	4.4	6.4	8-16	<1	<1	-	<1	<1	-
21 day	<2	<2	-	<2	2.4	14-	<1	<1	-	<1	<1	-

*ST = minimum and maximum survival time (Days) of animals that died after virus infection

Table II. TACARIBE VIRUS - LIVE ANTIGEN

VIRUS	CHALLENGE DOSE LD_{50}	CHALLENGE DOSE ID_{50}	EX NORMAL FEMALE HAMSTERS Deaths/T	EX NORMAL FEMALE HAMSTERS %	EX TACARIBE IMMUNE HAMSTERS Deaths/T	EX TACARIBE IMMUNE HAMSTERS %
TACARIBE	80	-	19/20	95	0/12	0
	800	-	22/22	100	3/17	19
JUNIN	0.1	1	7/37	19	5/28	18
	1	10	10/24	42	3/16	19
MACHUPO	1	19	9/21	43	10/21	48
	10	90	10/13	77	16/18	89

PROCEDURE:

1. Inoculate 14 day old female hamsters
2. Breed at 45 days of age
3. Challenge litters of suckling hamsters at 4 days of age

Table III. CHLOROFORM INACTIVATED MACHUPO VIRUS ANTIGEN

CHALLENGE MACHUPO VIRUS PFU Dose	EX NORMAL FEMALES			EX FEMALES AFTER 1 INOCULATION		
	Deaths/T	%	AST Days	Deaths/T	%	AST Days
1000	16/16	100	9.5	9/10	90	9.7
100	21/24	87	11.0	14/20	70	10.3
10	17/24	81	11.5	15/19	79	10.9

PROCEDURE:

1. Inoculate 14 day old female hamsters
2. Breed at 45 days of age
3. Challenge litters of suckling hamsters at 4 days of age.

Fig. 1. MACHUPO VIRUS GROWTH CURVE IN MA-111 CELL CULTURES

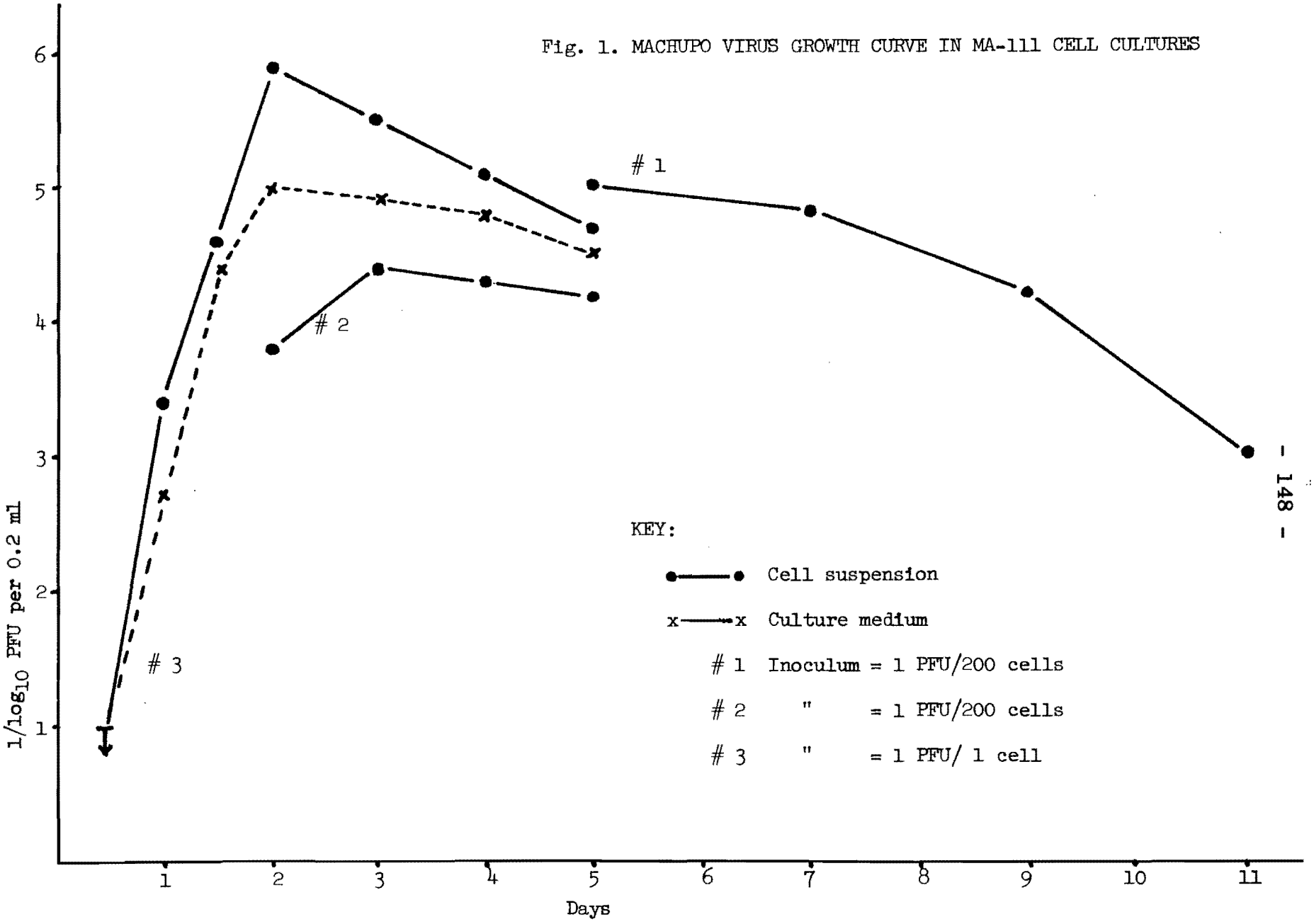
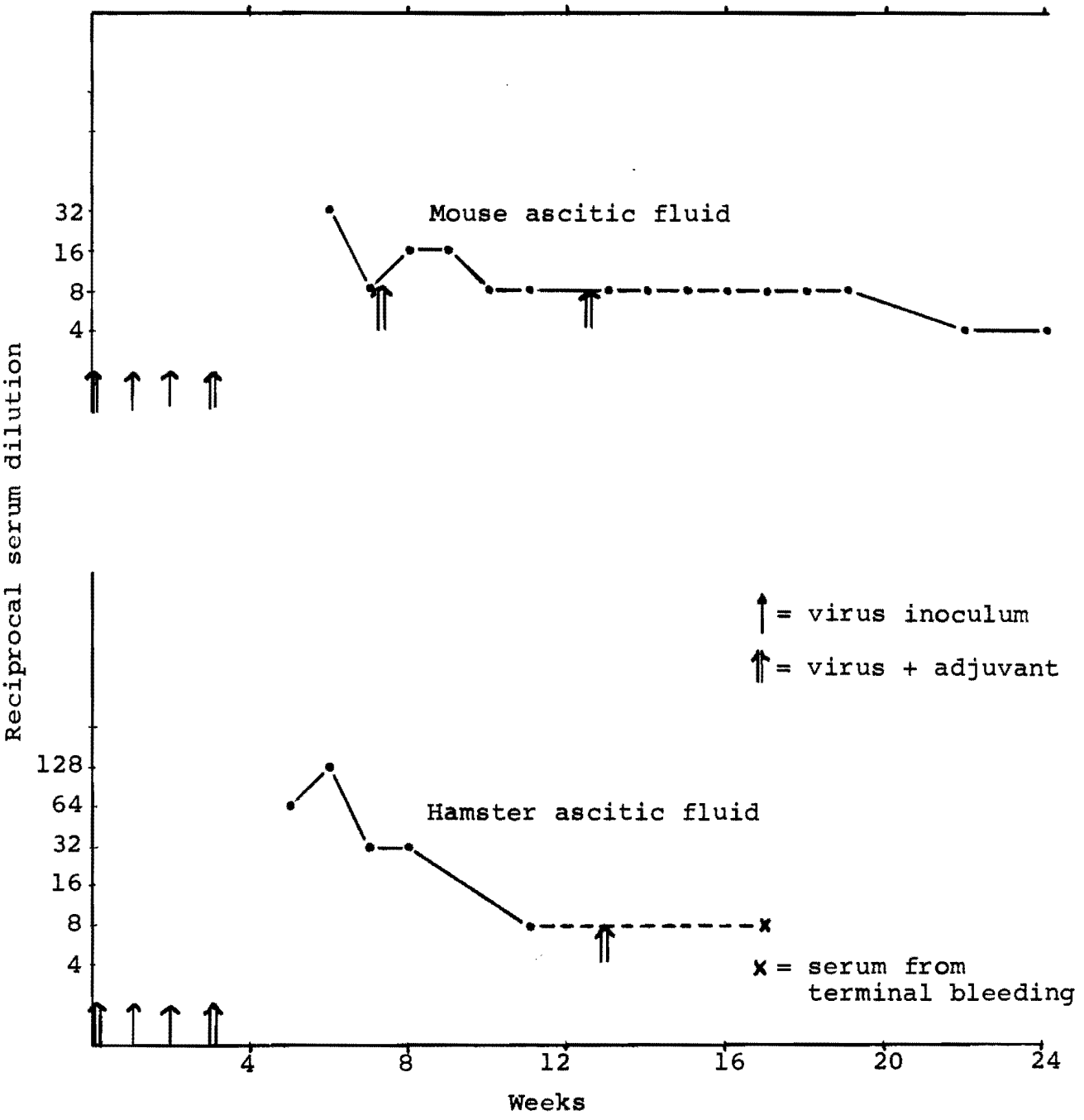


Figure 2. Complement Fixing Antibody Response After Intraperitoneal Inoculation Of Machupo Virus.



REPORT FROM DR. ARTHUR N. GORELICK
VIRUS AND RICKETTSIA DIVISION*
U.S. ARMY BIOLOGICAL LABORATORIES, FT. DETRICK, MARYLAND

Studies on Machupo virus are being conducted in this laboratory by Dr. Peter J. Gerone as part of a cooperative effort with the Laboratory of Tropical Virology and the Middle America Research Unit of NIH. Attempts at virus isolation from a fatal case of Bolivian hemorrhagic fever are reported here. Peripheral blood, bone marrow, and urine collected during the course of illness and tissues obtained at autopsy were examined for presence of virus. No virus was demonstrated in brain, spleen, kidney, lymph node, or liver tissue when these specimens were inoculated IP and IC into 3- to 7-day-old suckling hamsters. Peripheral blood specimens obtained on days 10 and 12 of illness were also negative when tested in suckling hamsters.

A specimen of urine obtained on the 14th day of illness was inoculated IP into a young adult hamster. The hamster showed signs of CNS disease 18 days postinoculation and was sacrificed the following day. Passage of brain tissue from this hamster yielded a virus that has been identified as Machupo virus by SN tests in hamsters, plaque-neutralization, and CF tests. The plaque-neutralization test was performed by Dr. Wiebenga, LTV, NIH. The CF tests were carried out by Dr. Johnson, MARU, and confirmed in our own laboratory.

A bone marrow aspirate was obtained on the 12th day of illness and cultured *in vitro* by Dr. Thomas J. Smith of the U.S. Army Medical Unit, Ft. Detrick. The technique employed was that described in detail elsewhere (Smith et al., Proc. Soc. Exp. Bio. Med., in press). Marrow cells were incubated *in vitro* as monolayer cultures of marrow alone (BM), and marrow in combination with either L cells, monkey heart (BM-MH) cells (Salk), or African green monkey kidney (BM-GMK) cells. Supernatant fluid was harvested from the culture of marrow cells alone at 24 hours, and from the other cultures at 24 hours, 72 hours, and 7 days. Fluids were tested for viral content by inoculation of suckling hamsters. Cell cultures were negative after 24 hours' incubation, but virus was demonstrated in supernatant fluids from all cultures tested at 72 hours, and in fluids from BM-MH and BM-GMK cell cultures at 7 days (Table 1). Virus recovered from the BM-GMK (7 day) culture has been identified as Machupo virus by CF and SN tests.

TABLE 1. VIRUS TITERS* FROM BONE MARROW CELL CULTURE SUPERNATANTS

Cell Culture	Harvest Time (Hours)		
	24	72	168
BM alone	0	--	--
BM + L	0	1.9	0
BM + GMK	0	trace	4.0
BM + MH	0	1.7	4.6

* Log_{10} S. hamster LD_{50} per ml.

The value of the urine specimen for isolation of virus has been shown previously (Johnson, personal communication). As mentioned above, no virus was demonstrated in peripheral blood on the day the marrow aspirate was obtained. Thus, the technique of in vitro cultivation of marrow favored the multiplication of, and subsequent demonstration of, virus at a time when viremia was not evident. These preliminary data indicate that culture of marrow in vitro is of value in isolating this virus relatively late in the course of the disease.

*In conducting the research reported herein, the investigator(s) adhered to "Principles of Laboratory Animal Care", as established by the National Society for Medical Research.

REPORT FROM DR. CARLOS SANMARTIN
UNIVERSIDAD DEL VALLE FACULTAD DE MEDICINA
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From July 5th to 11th, 1963, a trip was made to Choco to visit the area through which the Panamerican highway will pass. One of the localities included was Riosucio, on the lower Atrato River, and here a grab sample of 44 blood specimens was collected from local people. When tested in hemagglutination-inhibition against the Venezuelan equine encephalitis virus, 22 showed antibodies; only 2 had a titer of 1:10; the other 20 ranged from 1:80 to 1:1280 or above (geometric mean 1:350 or higher). This would indicate, according to our previous experience, that specific and recent antibodies for the Venezuelan virus are present in that geographical spot which becomes another recognized endemic area for this agent. In the accompanying table are the results of the mentioned tests.

Table I

Hemagglutination-inhibition tests with Venezuelan equine encephalitis virus and human sera collected in Riosucio, July, 1963

Age (years)	Male	Female	Total
1 - 4	-	0/1	0/1
5 - 9	1/6	-	1/6
10 - 14	5/13	0/3	5/16
15 - 19	0/1	3/3	3/4
20 - 29	2/2	0/4	2/6
30 - 39	6/6	2/2	8/8
40 - 49	1/1	1/1	2/2
50 or more	1/1	-	1/1
TOTAL	16/30	6/14	22/44

In the fractions the denominator indicates the number of sera tested and the numerator the number of positive

Table II

There were 24 virus isolations from mosquitoes captured at the Rio Raposo field station, from April through November 1962. The station is located in the Pacific lowland rain forest. The following table summarizes these isolations made by baby mouse inoculation.

Raposo River, 1962

Virus isolations from mosquitoes captured with human bait

Pool No.	Date of capture	Mosquito identification	No. in the pool	Virus identity
160	May 18-20	Mansonia arribalzagae	129	Group A
164	May 18-29	Psorophora ferox	30	Group A
165	May 18-29	Trichoprosopon spp.	137	Wyeomyia complex
175	May 29	Wyeomyia spp.	530	Una
178	May 29 June 4	Aedes serratus	78	Group A
284	May 21-30 June 4-6	Psorophora lutzi	24	Una
285	May 30 June 4-6	Psorophora ferox	32	Una
288	May 30 June 4-6	Trichoprosopon spp.	168	?
306	June 6	Wyeomyia spp.	302	Wyeomyia complex
316	June 12	Trichoprosopon spp.	74	Wyeomyia complex
317	June 13	Trichoprosopon spp.	34	Wyeomyia complex
319	May 16 June 10	Psorophora ferox	14	Una
340	May 21	Aedes serratus	37	Una
356	June 19	Trichoprosopon spp.	77	Wyeomyia complex
428	July 3	Wyeomyia spp.	300	Wyeomyia complex
568	July 24	Wyeomyia aporonoma	43	Wyeomyia complex
597	July 30	Limatus spp.	56	Wyeomyia complex
602	July 31 Aug. 6	Anopheles neivai	246	Guaroa
631	Aug. 8	Trichoprosopon spp.	104	Wyeomyia complex
632	Aug. 7	Anopheles neivai	102	Guaroa
812	Oct. 23-29	Limatus spp.	21	Wyeomyia complex
813	Oct. 23-29	Psorophora dimidiata	10	Wyeomyia complex
816	Oct. 22-23	Trichoprosopon leucopus	14	Wyeomyia complex
820	Oct. 22-23	Anopheles sp.	11	Wyeomyia complex

REPORT FROM DR. ENRIQUE PRIAS-LANDINEZ
AND DR. CARLOS BERNAL-CUBIDES
ARBOVIRUS LABORATORY, INSTITUTO NACIONAL DE SALUD
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From November to December 1963, a serologic survey was carried out at Guajira Peninsula, where an epidemic of VEE took place late in 1962. Two hundred twenty-five human sera were studied in the HI test, against a VEE antigen.

The total results indicated 48.4% positive reactors. Of eighty-six sera collected from people who stated that they were sick (VEE) during the epidemic, 66.3% yielded positive reactions. In contrast, only 37.4% positives were found among one hundred thirty-nine sera from people who denied being sick at that time (Table I). Most of the HI titers ranged between the 1:160 to the 1:1280 dilutions.

The results, according to jurisdiction (Table 2) showed fewer positive reactors at Puerto Estrella and Nazareth, small villages outside the epidemic area.

Likewise, the proportion of positive reactors was lower among fifty-one human sera collected during June 1964, at the towns of Dibulla, Fonseca, and Tomarrazon, also outside of the epidemic area.

The results in the HI test, using as antigen the French neurotropic strain of yellow fever virus, with sera of inhabitants of the Northeastern Zone of Guajira Peninsula, showed one positive out of fifty examined. The proportion for the Central Zone was twenty-nine out of one hundred seventy-five. And the corresponding to the Southwestern Zone was sixteen out of fifty-one sera.

The HI titers of the sera tested with the above mentioned Group B antigen usually ranged between the 1:10 and the 1:80 dilutions.

The Guajira Peninsula roughly can be divided into three major zones: the Northeastern, which is desertic of very scanty xerophilic vegetation and presenting some low hills,

called "serranias". Fresh water is almost absent and the area is poorly inhabited.

The Central Zone is flat desertic land, slightly richer in xerophilic vegetation; fresh water is difficult to obtain, and then only from some temporary wells. Small goat and donkey herds predominate; and the human population is mostly represented by nomadic indians.

The Southwestern Zone is of the tropical dried forest type with interspersed grassland areas; fresh water is relatively abundant. There are some flourishing cattle ranches; and the population is mostly represented by sedentary white people.

In the Guajira Peninsula, the limiting factors are the trade winds, the low annual rainfall, and the temporature above 24°C.

The processing of mammalian and enthomologic materials collected in the Guajira, through successive visits, is underway and will be reported later.

TABLE I

Results in the HI test using an VEE antigen obtained with human sera collected in Guajira, Colombia, November-December 1963.

Age (years)	Sick			Not sick			Totals		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
1-4	0/1[x]	-	0/1	-	-	-	0/1	-	0/1
5-9	7/10	4/8	11/18	4/8	1/5	5/13	11/18	5/13	16/31
10-14	5/7	1/3	6/10	2/12	3/12	5/24	7/19	4/15	11/34
15-19	4/6	5/9	9/15	4/13	2/21	6/34	8/19	7/30	15/49
20-29	1/2	5/8	6/10	3/9	6/16	9/25	4/11	11/24	15/35
30-39	4/4	8/12	12/16	6/9	3/10	9/19	10/13	11/22	21/35
40-49	4/4	4/5	8/9	4/5	8/11	12/16	8/9	12/16	20/25
50 o más	3/5	2/2	5/7	3/3	3/5	6/8	6/8	5/7	11/15
Total	28/39	29/47	57/86	26/59	26/80	52/139	54/98	55/127	109/225
%	71.8	61.7	66.3	44.1	32.5	37.4	55.1	45.3	48.4

x Number of positives/number examined.

T A B L E II

Results in the HI test, using a VEE antigen, with human sera collected in Guajira, and according to Jurisdiction. November-December 1964.

Jurisdiction	Posit. examined	%
Central Zone		
Riohacha (6)[x]	16/33	48.5
Maicao (9)	35/63	55.5
Carraipía (2)	12/26	46.1
El Pájaro (6)	16/19	84.2
Manaure (1)	6/9	66.6
Uribia (3)	14/19	73.7
Cabo de la Vela	2/6	33.3
Northeastern Zone		
Bahía Honda	5/12	41.6
Puerto Estrella	1/15	6.6
Nazareth	2/23	8.7
Southwestern zone		
Dibulla [xx]	1/9	10.0
Fonseca [xx]	2/10	20.0
Tomarrazón [xx]	4/32	12.15

x - Numbers in parenthesis are number of settlements under jurisdiction where people were bled.

xx- In these localities the bleeding took place in June 1964.

REPORT FROM DR. G.H. BERGOLD
INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS (IVIC)
CARACAS, VENEZUELA

VEE Outbreaks: At the end of June and during July, many newspaper articles claimed an increased number of encephalitis cases. Several fatalities were reported in the states of Monagas and Sucre. No samples have been received in this department, but Dr. H. Fossaert informed me that VEE cases were isolated from Carupano, and that he was able to isolate VEE from 7 out of 10 cases in Caripe, north of the State of Monagas. The samples of Caripe were particularly interesting because they came from a location of comparative high altitude. According to Dr. Fossaert's opinion, another virus may be active in the region of Caripe, and he believes that it might be dengue on the basis of an HI test of one sample from Carupano and one from Margarita. Furthermore, Dr. Fossaert says that baby mice inoculated with acute-phase sera look suspiciously ill from the 7th day on. Mr. O. Suarez and myself will visit the affected area on July 23rd, to catch mosquitoes and collect serum samples.

Serological investigations: Finally all sera collected in the last years in different parts of Venezuela were investigated partially with the macro method, and all of them with the microtiter technique. This was carried out by Dr. A. Morales, with the kind cooperation of Dr. L. Spence, from Trinidad Regional Virus Laboratories. The results are presently summarized. It is interesting that in the headwaters of the Orinoco, antibodies against VEE, Mayaro, Una, Cache Valley, Manzanilla, Caraparu, Dengue, Ilheus, Yellow Fever, St. Louis, etc., were found in human sera. In sera of monkeys, rats, and reptiles, antibodies against VEE, Una, Ilheus, Yellow Fever, Semliki, Caraparu, etc., were detected. All these results are presently summarized for publication. The serological investigation of samples taken in connection with the VEE outbreaks of the past years were also terminated and previous findings were confirmed. For instance, 10 out of 27 goats had antibodies against VEE. A few goats had antibodies against Ilheus, Yellow Fever, and St. Louis, etc. Of a total of 67 bird samples investigated, only one had antibodies against VEE, two against St. Louis, one against Ilheus, and one against Cache Valley. All these results are presently summarized for publication.

The several years' research for an overlay medium that will support plaque formation of practically all arboviruses is finally terminated. After using about 18,000 disposable tissue culture flasks (Falcon), an overlay based on that of Miles was developed using 7% Bovalbumine V Fraction (NBC) instead of serum, and adding 0.25 ug/ml of total overlay. For practically all viruses of the B group, addition of Cortisol is necessary for good plaque formation. As is generally known, the type of Agar is of great importance too. The German Agar was found to be suitable, but on the kind suggestion of Dr. Porterfield, a South African and a French Agarose were tried. The French Agarose (L'Industrie Biologique Francaise, Quai du Moulin de Cage, Genevilleirs, France) was found to be the best. Generally the addition of Cortisol is improving the appearance of plaques of all viruses, although this is not necessary for several. It is of interest that the addition of Cortisol to the French Agarose in the presence of DEAE is toxic to BHK21 cells. Of further importance is the reduction of Agar concentration to 0.5%/total overlay. Some details of time of appearance, shape of plaques, as well as titers, are summarized in the attached table. With this improved overlay, we can readily obtain plaques of all arboviruses available to us, except of the Tacaribe (BHF) group and Caraparu. Since the French Agarose (without DEAE) plus Cortisol is improving plaques of all viruses, we are using this type of overlay routinely. Caraparu (Trinidad strain) will occasionally produce plaques, but the appearance is not reliable. Similarly, Tacaribe shows plaque-like formations on the 6th day, but counting is difficult. These results are presently summarized for publication.

By the kind cooperation of Dr. Vincent from the MBA, we have received several new cell lines which are presently being investigated for their suitability for plaque formation of arboviruses, particularly the group C and BHF.

The fine structure of Cocal, Indiana, and New Jersey viruses of the vesicular stomatitis group were compared with the electron microscope. No obvious differences in the morphological structure could be found. There are some slight differences in the dimensions, but the general

appearance is the same for all three viruses. The electron microscopic investigation led to a speculative interpretation of the virus rods as a combination of filamentous helical structures. A manuscript on these findings is presently prepared for publication. Preliminary electron microscopic investigations of Guaroa virus indicate that this virus is spherical with a diameter of about 80 mu and contains several capsids.

Together with Mr. Suarez, the investigations of the development of VSV in mosquitoes are almost concluded. During these investigations, which were supported by a PHS grant, VSV particles were found in the intestine, thoracic ganglion and salivary glands. Considerable damage to the infected cells indicates that these are sites of multiplication of the virus. These findings are presently summarized for publication.

In response to our advertising in SCIENCE and NATURE, nine applications from virologists were received. Presently, negotiations are underway to fill the position previously occupied by Dr. Sellers.

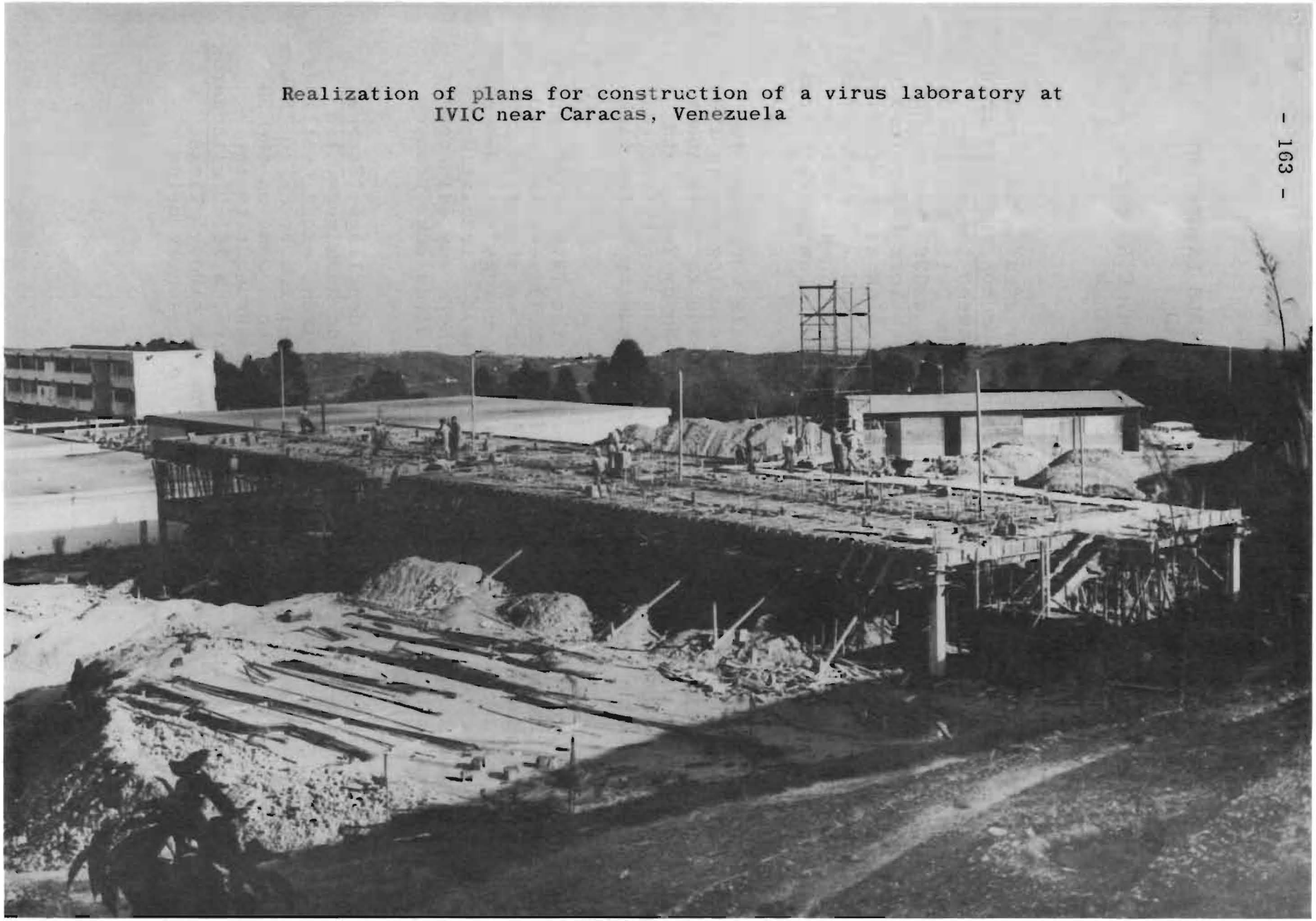
The construction of the new virus building is advancing according to schedule. Actually, the third and last floor is under construction. The new building will have the name of "Centro de Virologia 'Beauperthuy'".

LIST OF VIRUSES PRODUCING PLAQUES IN BHK 21 CELLS USING A MODIFIED OVERLAY

Virus	Plaque Formation: Days	Shape*	Diameter	Agar: Noble	Germ.	French	Addition of: DEAE	Cortisol	Titer: 10^{x}
Group A									
Una	3-4	R	0.5-3.0	+	+	+	no	no	5-6
EEE	2	R	1-3.0	+	+	+	no	no	8
WEE	2	R	1.0-4.0	+	+	+	no	no	8
VEE	1-2	R	3-5	+	+	+	no	no	8
Mayaro	2	R	0.5-4.0	+	+	+	no	no	7-8
Simliki	2	R	0.5-4.0	+	+	+	no	no	8
Group B									
Bussuguara	4	C	0.3-1.0	-	+	+	yes	yes	5-6
Dengue I	5	C-R	0.2-0.8	-	-	+	no	Yes	3
Dengue II	5	C	0.2-0.8	-	-	+	no	yes	3
Ilheus	3	R	1-3	+	+	+	yes	yes	6
St. Louis	3	R	0.5-3.0	+	+	+	yes	yes	6-7
YF 17D P	4	R	0.5-1.0	-	+	+	yes	yes	4
YF 17D C	4	R	0.5-1.0	-	+	+	yes	yes	5
FN	4	R	1.0-3.5	±	+	+	yes	yes	5
IVIC 1	4	R	0.5-1.5	-	+	+	yes	yes	5
IVIC 2	4	R	1.0-2.0	-	+	+	yes	yes	4
JSS	4	R	1.0-4.0	-	+	+	yes	yes	6-7
Manera	4	R	1.0-2.0	-	+	+	yes	yes	5
Bumyamwera									
Cache Valley	2-4	C-R	1.0-4.0	?	+	+	yes	no	6
Guaroa	3	C-R	0.5-2.0	+	+	+	yes	no	6
Kairi	4	C	0.2-1.0	?	+	+	yes	no	6
Wyeomyia	3	C-R	0.5-3.0	-	+	+	yes	yes	6
Guana									
Guana	2-4	R	0.5-1.5	?	+	+	yes	no	4-5
Bimiti	5	C	0.2-0.5	?	+	+	yes	no	5
Catu	4	C	0.2-0.8	?	±	+	yes	yes	5-6
Others									
Anophel A	4	R	0.5-3.0	+	+	+	yes	no	6
" B	4	C-R	0.5-1.0	-	±	+	yes	yes	4
Melao	4-5	R	0.5-2.0	?	+	+	yes	yes	5-6
Manzanilla	4-5	R	0.5-1.5	?	+	+	yes	no	4
Oropouche	3-4	C	0.5-1.0	?	+	+	yes	yes	4
Triniti	4-5	C	0.5-1.0	?	+	+	yes	no	4-5
VSV									
Indiana	1	R	0.5-3.0	+	+	+	no	no	8
New Jersey	1	R	0.5-2.0	+	+	+	no	no	5
Cocal	1	R	0.5-2.0	+	+	+	no	no	7

Foot note: * R= Round; C=Cirriform

Realization of plans for construction of a virus laboratory at IVIC near Caracas, Venezuela



REPORT FROM THE TRINIDAD REGIONAL VIRUS LABORATORY PORT-OF-SPAIN, TRINIDAD

Contributors: L. Spence, T.H.G. Aitken, C.B. Worth, A.H. Jonkers, and E.S. Tikasingh

Soldado Rock Tick Virus Studies:

In 1962, this laboratory isolated four strains of virus from bird-feeding ticks of the Ornithodoros capensis group, collected on Soldado Rock, halfway between the south-western tip of Trinidad and the Orinoco Delta country of Venezuela. The prototype strain (TRVL 42336) has been shown by the Rockefeller Foundation New York Laboratory to be related, if not identical, to a tick agent described by Hughes et al., (Am. J. Trop. Med. & Hyg., 13:118, 1964) from the Dry Tortugas Islands, Florida. These workers also report the recovery of the Dry Tortugas isolate from ticks in the Gulf of Baja, California.

Four expeditions at trimonthly intervals were made to Soldado Rock in 1963 and four more are being run in 1964. On these trips, an attempt is being made to bleed about 100 birds (mainly Sooty Terns, Brown Noddy Terns, and Grey-breasted Martins). Birds are subsequently banded. These bloods are being studied for immunity to the tick virus.

In 1963, three additional strains of the TRVL 42336 prototype virus and one new virus (TRVL 52214) were isolated from Soldado ticks. Also in that year we attempted to demonstrate circulating virus in baby chicks (six 3-week-old and three "wet" 1/2-day-old chicks). Circulating virus was not demonstrable, nor was there any evidence of neutralizing antibodies in the older birds (the younger birds still have to be tested).

In May 1964, a group of 33 Sooty Tern nestlings (Sterna fuscata)(8-12 days of age) from Soldado Rock were bled for circulating virus. Of these 33 birds, eight nestlings (24%) yielded a virus serologically related by CF to TRVL 42336. Further serology has not yet been done on the 33 bloods. Four of the tern chicks were brought to the laboratory and over a period of 10 days, 3,751 Ornithodoros ticks (mainly larvae) dropped from the birds. Ticks collected on the May expedition are presently being tested for virus.

The Use of Adjuvant and S.180 Cells in the Production of Hyperimmune Ascitic Fluids:

In 1962, we started a project to study the possible value of adjuvant in combination with Sarcoma 180 cells in the production of large amounts of high titered hyperimmune ascitic fluids using mice as the experimental animal. This report describes our procedure and summarizes our results.

Individual hyperimmune ascitic fluids were produced as follows: 0.1 ml. of a 10% suspension of virus together with 0.1 ml. of adjuvant was inoculated IP into five groups of mice (6 mice/group). Five inoculations were given at weekly intervals. Three days (plus or minus 1 day) prior to the fifth inoculation, 0.2 ml. of approx. 4% suspension of Sarcoma 180 cells was administered. Fluids were harvested in about 7-10 days after the last inoculation. At first, we used both complete and incomplete Freund's adjuvant, but these were abandoned early in the project when we found a mixture of one volume of Arlacel A and three volumes of Bayol F gave satisfactory results.

Some of our viruses are pathogenic for adult mice by the IP route. We therefore used a killed (with BPL) suspension of the virus and administered two preimmunizing shots on day 0 and day 2, followed by live shots on days 7, 14, 21, and 28. With our Caraparu-like virus, three preimmunizing shots were given on days 0, 2, and 7, followed by live shots on days 14, 21, 28, and 35.

Hyperimmune ascitic fluids have been produced for all our prototype viruses (except for Guama group agents where hyperimmune sera and ascitic fluids are of little help in distinguishing the individual strains). In addition, nine hyperimmune group ascitic fluids were produced using polyvalent vaccines. Such groups were kept as natural units where possible, e.g., all members of serological group A were kept as one unit, but nine viruses which were not related were arbitrarily placed in three groups.

The entire series of ascitic fluids have been tested with homologous and heterologous antigens in CF and HI tests. In addition, N tests were done on a selected number of ascitic fluids to study their neutralizing capacities. The results are presented in Tables 1-6. Table 1 lists

the viruses to which ascitic fluids were prepared together with the volumes obtained for each virus (30 mice used for each virus except Melao where 60 mice were used) and a comparison of CF titers of the fluids and antisera. The great variation of the volumes of ascitic fluid obtained from each group of 30 mice is at once apparent. Volumes as low as 17 ml. and high as 98 ml. were obtained with an average of 1.3 ml./mouse for the entire series. However, it should be pointed out that this average was based on the entire lot of 810 mice which were used in the experiments and not on the number of mice surviving at the time the fluids were harvested. There are often spontaneous deaths after each inoculation, particularly when the Sarcoma cells were administered. (Note: We are now using a slow strain of S.180 cells obtained through the courtesy of Dr. W.G. Downs, which, when used as a 10% suspension, develops more slowly in mice, with production of considerable volumes of ascitic fluids over a longer period of time, permitting one to obtain 10-20 ml. per mouse).

The CF titers of the hyperimmune ascitic fluids and sera taken from the same group of mice are quite comparable as shown in Table 1. Titers of the ascitic fluids ranged from 1:32 to $1:1024^{+}$ while that of the sera ranged from 1:8 to $1:1024^{+}$. Only six ascitic fluids and five sera did not give titers greater than 1:64.

Table 2 gives the results of homologous and heterologous CF tests with the 26 hyperimmune ascitic fluids and antigens. Specific reactions were obtained only with homologous systems and with members of the same serological groups. The original Mayaro hyperimmune ascitic fluid gave a number of non-specific reactions with heterologous antigens, but such reactions were not seen with the new ascitic fluid (#45719).

HI results with homologous and heterologous hemagglutinins are presented in Table 3. Again we see high titers, ranging from 1:320 to $1:5120^{+}$. Cross-reactions are here more marked than in the CF tests, but these occur only within serological groups.

Neutralization tests were also performed on 14 of the ascitic fluids in an attempt to study their neutralizing capacities. The results are presented in Table 4. Titrations were done in suckling mice i.c. and the titers are expressed as the logarithm to the base ten of the

reciprocal of the 50 per cent mortality endpoint. Log neutralization indices as shown in Table 4 ranged from 3.7 to 7.4. The table also shows that titrations in normal ascitic fluids and BAD (when done) are quite comparable, thus illustrating the point that the ascitic fluids are not necessarily detrimental to viruses per se. Also, deaths attributable to the ascitic fluids were not seen in the N tests.

Both CF and HI results with homologous and heterologous antigens and group ascitic fluids are presented in Tables 5 and 6. Again, we see specific reactions of homologous systems. Although titers in a few cases were disappointing, particularly with Group A and Guama group hyperimmune ascitic fluids, they were nevertheless sufficiently high to place any one of the 30 agents shown in Table 2 in its respective group. We have already utilized the 10 group ascitic fluids successfully in the rapid identification of our 1963 isolates both in CF and N tests.

In summarizing, the following points emerge from this study:

1) The use of adjuvant and S.180 shows great potential in the production of large quantities of high-titered hyperimmune ascitic fluids.

2) Hyperimmune ascitic fluids so produced are specific for homologous strains of viruses or closely related strains and are without non-specific reactions.

Table I

List of hyperimmune ascitic fluids prepared to TRVL prototype viruses using adjuvant and Sarcoma 180 cells together with a comparison of homologous CF titers of ascitic fluids and antisera.

Virus	Volume of ascitic fluid (in ml)	CF titer of ascitic fluid	CF titer of serum
EEE (TRVL 24443)	28	512*	512*
WEE (TRVL 25717)	50	256	512
VEE (TRVL 28215-1)	36	32	32
Mayaro (TRVL 15537)	30	256	ND
Una (TRVL 41308)	47	256	ND
Ilheus (TRVL 3089)	40	256	512
St. Louis (TRVL 9464)	28	256	256
Yellow Fever (TRVL 2942)	40	256	512
Dengue II (TRVL 1751)	24	128	256
Caraparu (TRVL 34053-1)	98	512	256
TRVL 18462	48	256	256
Oropouche (TRVL 9760)	30	256	256
Manzanilla (TRVL 3587)	50	512	ND
Melao (TRVL 9375)	78	512	512
Cache Valley (TRVL 20659)	30	512	1024+
Kairi (TRVL 8900)	25	1024+	1024+
Wyeomyia (TRVL 8349)	50	1024	ND
Triniti (TRVL 7994)	49	16	32
TRVL 8762	18	32	8
TRVL 9223	45	32	128
Tacaribe (TRVL 11573)	56	32	16
Cocal (TRVL 40233)	34	64	256
TRVL 42336	30	256	128
TRVL 10076	17	256	512
TRVL 42520-1-5	77	128	128
TRVL 39316-1-5	25	128	64

*Reciprocal of ascitic fluid and serum titers giving more than 50% fixation.

+Denotes endpoint was not reached.

Table II

Results of CF homologous and heterologous tests done on 26 hyperimmune ascitic fluids which were prepared to TRVL prototype viruses using adjuvant and Sarcoma 180 cells.

ANTIGENS	Optimum dilution of antigen	HYPERIMMUNE ASCITIC FLUID: EEE (#59335)	WEE (#59553)	VEE (#59992)	Mayaro (#45719)	Una (#45157)	Ilheus (#49126)	St. Louis (#48702)	Yellow Fever (#47742)	Dengue II (#48700)	Caraparu (#45324)	TRVL 18462 (#48290)	Cache Valley (#48870)	Kairi (#48868)	Nyeomyia (#45142)	Manzanilla (#45114)	Oropouche (#49671)	Melao (#48935)	Trinitl (#51288)	TRVL 8762 (#51049)	TRVL 9223 (#49673)	Tacaribe (#54316)	Cocal (#53498)	TRVL 42336 (#49847)	TRVL 10076 (#51047)	TRVL 42520-1-5 (#49128)	TRVL 39316-1-5 (#51045)
EEE	4	512*	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WEE	4	16	256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VEE	4	4	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mayaro	4	0	0	0	256	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Una	4	0	0	0	4	256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ilheus	16	0	0	0	0	0	256	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
St. Louis	16	0	0	0	0	0	16	256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yellow Fever	4	0	0	0	0	0	0	0	256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dengue II	4	0	0	0	0	0	0	0	0	128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caraparu	4	0	0	0	0	0	0	0	0	0	512	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TRVL 18462	16	0	0	0	0	0	0	0	0	0	0	256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cache Valley	128	0	0	0	0	0	0	0	0	0	0	0	512	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kairi	8	0	0	0	0	0	0	0	0	0	0	0	0	1024+	16	0	0	0	0	0	0	0	0	0	0	0	0
Nyeomyia	32	0	0	0	0	0	0	0	0	0	0	0	0	0	1024	0	0	0	0	0	0	0	0	0	0	0	0
Manzanilla	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	512	0	0	0	0	0	0	0	0	0	0	0
Oropouche	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256	0	0	0	0	0	0	0	0	0	0
Melao	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	512	0	0	0	0	0	0	0	0	0
Trinitl	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0
TRVL 8762	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0	0	0
TRVL 9223	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0	0
Tacaribe	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0
Cocal	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	64	0	0	0	0
TRVL 42336	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256	0	0	0
TRVL 10076	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256	0	0
TRVL 42520-1-5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	128	0
TRVL 39316-1-5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	128

* Reciprocal of ascitic fluid dilution giving more than 50% fixation.

\+ Denotes endpoint was not reached.

Table III

Results obtained with homologous and heterologous haemagglutinins and hyperimmune ascitic fluids prepared with adjuvant and S.180 cells (adjusted to 4 HA units)

Hyperimmune ascitic fluids \ Antigens	EEE	WEE	VEE	Mayaro	Una	Ilheus	SLE	YF	Dengue	Caraparu	Cache Valley	Manzanilla
EEE (#45935)	2560*	80	40	10	0	0	0	0	0	0	0	0
WEE (#45953)	80	2560	40	0	0	0	0	0	0	0	0	0
VEE (#45992)	40	0	320	0	0	0	0	0	0	0	0	0
Mayaro (#45719)	320	320	80	1280+	80	0	0	0	0	0	0	0
Una (#45157)	0	160	20	20	320	0	0	0	0	0	0	0
Ilheus (#49126)	0	0	0	0	0	2560	2560	320	320	0	0	0
SLE (#48702)	0	0	0	0	0	1280	5120+	640	640	0	0	0
YF (#47742)	0	0	0	0	0	320	1280	640	320	0	0	0
Dengue (#48700)	0	0	0	0	0	320	640	160	1280	0	0	0
Caraparu (#45324)	0	0	0	0	0	0	0	0	0	320	0	0
Cache Valley (#48870)	0	0	0	0	0	0	0	0	0	0	2560	0
Manzanilla (#45114)	0	0	0	0	0	0	0	0	0	0	0	320

* Reciprocal of ascitic fluid titer

+ Denotes endpoint was not reached

Table VI

HI RESULTS WITH 10 GROUP HYPERIMMUNE ASCITIC FLUIDS AND 12 TRVL ANTIGENS. (RESULTS ADJUSTED TO 4 HA UNITS).

	EEE	VEE	WEE	MAYARO	UNA	ILH	SLE	DENGUE	YF	CARAPARU	CV	MANZ.
Group A (48151)	320*	80	160	320	640	0	0	0	0	0	0	0
Group B (49845)	0	0	0	0	0	5120	640	1280	1280+	0	0	0
Group C (49453)	0	0	0	0	0	0	0	0	0	320	0	0
Guama (49257)	0	0	0	0	0	0	0	0	0	0	0	0
Bunyamwera (48865)	0	0	0	0	0	0	0	0	0	0	1280+	0
Simbu (48930)	0	0	0	0	0	0	0	0	0	0	0	640
California (48935)	0	0	0	0	0	0	0	0	0	0	0	0
Ungrouped-I (49032)	0	0	0	0	0	0	0	0	0	0	0	0
Ungrouped-II (49151)	0	0	0	0	0	0	0	0	0	0	0	0
Ungrouped-III (49053)	0	0	0	0	0	0	0	0	0	0	0	0

*Reciprocal of ascitic fluid titer.

+Denotes endpoint was not reached.

Table IV

N tests done on 14 hyperimmune ascitic fluids prepared by using adjuvant and S. 180 cells. (All titrations in suckling mice, i.c.)

Virus	Pool Titer (BAD)	Titer in Normal Ascitic Fluid	Titer in Hyperimmune Ascitic Fluid	LNI
EEE	8.5	8.5	<1.5	>7.0
WEE	ND	8.9	<1.5	>7.4
VEE	7.5	7.5	2.7	4.8
Mayaro	ND	8.0	<1.5	>6.5
Una	ND	6.9	<1.5	>5.4
Ilheus	ND	>8.3	4.6	>3.7
St. Louis	ND	7.9	4.0	3.9
Dengue	ND	6.6	2.5	4.1
Yellow fever	ND	7.5	<1.5	>6.0
Caraparu	6.9	6.4	<1.5	>4.9
Manzanilla	7.5	6.5	<1.5	>5.0
Cache Valley	8.0	6.7	2.2	4.5
Triniti	5.5	5.3	<1.5	>3.8
Aruac	6.7	ND	<1.5	>5.2

ND = Not done.

LNI = Logarithm neutralization index.

Table V

CF results with 10 group hyperimmune ascitic fluids and 30 TRVL antigens.

	ASCITIC FLUID TITERS									
ANTIGENS	Group A #48151	Group B #49845	Group C #49453	Group Bunyamwera #48865	Group Simbu #48930	Group California #48935**	Group Guama #49257	Ungrouped Viruses-I #49032	Ungrouped Viruses-II #49150	Ungrouped Viruses-III #49053
Group A										
EEE	32*	0	0	0	0	0	0	0	0	0
WEE	32	0	0	0	0	0	0	0	0	0
VEE	32	0	0	0	0	0	0	0	0	0
Mayaro	32	0	0	0	0	0	0	0	0	0
Una	16	0	0	0	0	0	0	0	0	0
Group B										
Ilheus	0	128	0	0	0	0	0	0	0	0
SLE	0	64	0	0	0	0	0	0	0	0
YF	0	128	0	0	0	0	0	0	0	0
Dengue II	0	32	0	0	0	0	0	0	0	0
Group C										
Caraparu	0	0	128	0	0	0	0	0	0	0
TRVL 18462	0	0	256	0	0	0	0	0	0	0
Group Bunyamwera										
Cache Valley	0	0	0	512	0	0	0	0	0	0
Kairi	0	0	0	128	0	0	0	0	0	0
Wyeomyia	0	0	0	512	0	0	0	0	0	0
Group Simbu										
Manzanilla	0	0	0	0	256	0	0	0	0	0
Oropouche	0	0	0	0	256	0	0	0	0	0
Group California										
Melao**	0	0	0	0	0	512	0	0	0	0

*Reciprocal of ascitic fluid dilution giving more than 50% fixation

**Only one member of this group isolated in Trinidad.

Table V (cont'd)

ASCITIC FLUID TITERS

ANTIGENS	Group A #48151	Group B #49845	Group C #49453	Group Bunyamwera #48865	Group Simbu #48930	Group California #48935**	Group Guama #49257	Ungrouped Viruses-I #49032	Ungrouped Viruses-II #49150	Ungrouped Viruses-III #49053
Group Guama										
Bimiti	0	0	0	0	0	0	64	0	0	0
Guama	0	0	0	0	0	0	256	0	0	0
Catu	0	0	0	0	0	0	16	0	0	0
TRVL 26668	0	0	0	0	0	0	8	0	0	0
Ungrouped Viruses-I										
Triniti (TRVL 7994)	0	0	0	0	0	0	0	256	0	0
TRVL 8762	0	0	0	0	0	0	0	16	0	0
TRVL 9223	0	0	0	0	0	0	0	256	0	0
Ungrouped Viruses-II										
Tacaribe	0	0	0	0	0	0	0	0	8	0
TRVL 40233	0	0	0	0	0	0	0	0	128	0
TRVL 42336	0	0	0	0	0	0	0	0	128	0
Ungrouped Viruses-III										
TRVL 10076	0	0	0	0	0	0	0	0	0	64
TRVL 42520-1-5	0	0	0	0	0	0	0	0	0	512
TRVL 39316-1-5	0	0	0	0	0	0	0	0	0	64

*Reciprocal of ascitic fluid dilution giving more than 50% fixation.

**Only one member of this group isolated in Trinidad.

REPORT FROM DR. ROBERT E. SHOPE
BELEM VIRUS LABORATORY, INSTITUTO EVANDRO CHAGAS
BELEM, BRAZIL

During the first half of 1964, mosquitoes captured on mouse and chicken bait in the Instituto Agronomico do Norte forest were identified while alive and liberated in large holding cages in the forest to attempt to obtain oviposition for subsequent identification of larvae, pupae, and adults of both sexes. Families of Swiss mice were placed in the cages to provide a blood meal and were observed later for illness.

Between February 24 and June 30, 2846 Culex (M) taeniopus females were released in the cage and 68 families of mice exposed. Guama virus was isolated from blood of a mother mouse exposed on April 14.

Between January 30 and June 30, 2860 female mosquitoes of a species morphologically similar to what has been called Culex no. 9 of Trinidad, were released in another cage. One hundred nine families of mice were exposed during this period and Oriboca virus was isolated from a baby mouse exposed on May 9.

Two virus transmissions from different species of naturally infected Culex mosquitoes were thus observed. This sentinel mouse-captive mosquito technique offers a relatively simple way to demonstrate the naturally infected vector if it can be identified while alive.

REPORT FROM DR. OSCAR DE SOUZA LOPES,
INSTITUTO ADOLFO LUTZ, AND DR. O.P. FORATINI,
SCHOOL OF HYGIENE AND PUBLIC HEALTH, SAO PAULO, BRAZIL

There were some changes in our field stations as it was described in Infoexchange No. 4. After working in places with no rewarding results, we decided to concentrate our efforts in two places. The first, "Cotia", is the field station where we started working in 1961. There, we isolated a virus, which remains ungrouped. It is a secondary growth forest reserve with two reservoirs to supply water to our city, located about 45 kilometers east from the laboratory. The second one was established in a huge forest reserve in a place called "Casa Grande",

about 100 kilometers west from the laboratory. There, we did some work in 1962 and it was described in the Info-exchange No. 4 as "Borecea". The work done in these two places is almost the same already described.

Last September we received a visit from Dr. T.H. Aitken from the Trinidad Regional Virus Laboratory, among other visitors. After his stay, an effort is being made to catch more mosquitoes, especially the nocturnal ones. We built some mosquito traps, which are not as productive as anticipated. The use of the Shannon Dawn Trap, with light bait, is the type that is giving more mosquitoes. The other baits are still being experimented with and the results will be presented on another occasion.

Cotia has been studied for the past three years. From there, we isolated many strains of a virus that we are calling "Cotia virus", always from sentinel mice. It is considered ungrouped up to now and it is described in the Arbovirus Catalogue as entry No. 125. Our work was confirmed by Dr. Robert E. Shope from the Belem Virus Laboratory.

Our mouse colony is being enlarged, giving us more animals to work with. So, we could begin to identify some isolates that were shown to be different from Cotia virus in CF tests and behavior in mice. The serological identifications are in progress and the results will be presented in another report.

With more animals, an effort is being made to prepare more homologous systems with the viruses isolated in the Americas. We received several strains from the Rockefeller Foundation Virus Laboratories and from the Belem Virus Laboratory. We intend to have most of these systems available by July in order to have precise identification of our isolates.

REPORT FROM A.S. PARODI AND CELIA COTO, FACULTAD DE MEDICINA, BUENOS AIRES

Immunization of guinea pigs against Junin virus (Argentine hemorrhagic fever) with Tacaribe virus:

Junin virus, the etiological agent of Argentine hemorrhagic fever (1), has been found to be antigenically related with Tacaribe virus by complement fixation (2), although cross neutralization in tissue culture has not been demonstrated.

Tacaribe virus isolated in Trinidad is pathogenic for baby mice inoculated by IC route, but we found that it is not pathogenic for guinea pigs by IM, IP, or IC route.

The guinea pig is the most susceptible animal to Junin virus. It is for this reason that it was not easy, up to the present, to immunize guinea pigs with Junin virus, although it was intended using virus inactivated by several ways, like U.V. rays, chemical agents, etc., inoculated with or without adjuvants.

Nine guinea pigs of 250 g. weight were inoculated with Tacaribe virus using 0.2 ml. of a 10% suspension of mice brain in phosphate buffer pH 7.4 by the IM route.

Another group of guinea pigs was inoculated with yellow fever vaccine and a third group of animals remained as a control, without any inoculation.

The inoculations were repeated two times more, fifteen days apart, and fifteen days after the last inoculation, all the animals, including the control, were challenged with a 0.2 ml. of 10^{-4} dilution of Junin virus, equivalent to 1000 LD_{50} for guinea pigs.

All the animals except those inoculated with Tacaribe virus, died between the 13th and 16th days of the last inoculation (Table 1).

As we can see with these results, the Tacaribe virus gave a very good protection against Junin virus.

Experiments in course show that with only one inoculation with Tacaribe virus, the guinea pigs are protected against 1000 LD_{50} of Junin virus.

References:

1. A.S. Parodi, D.J. Greenway, H.R. Rugiero, E. Rivero, M. Frigerio, J.M. de la Barrera, N. Mettler, F. Garzon, M. Boxaca, L. Guerrero, N. Nota. Dia Medico, 30, 2300 (1958).

2. N. Mettler, J. Casals, R. Shope. Amer. J. of Trop. Med. and Hyg., 12, 647 (1963).

3. M. Boxaca, A.S. Parodi, H. Rugiero, R. Blay, Rev. Soc. Arg. Biol., 37, 170 (1961).

Table I

RESISTANCE OF GUINEA PIGS AGAINST JUNIN VIRUS INFECTION AFTER INOCULATION OF TACARIBE VIRUS AND YELLOW FEVER VACCINE

Virus Inoculated For Immunization	No. Animals	Challenged with 1000 LD_{50} of Junin Virus No. of Deaths
Tacaribe virus	9	0
Yellow Fever vaccine	9	9
Control	8	8

EDITORIAL NOTE

It is with regret that Issue Number 10 of the Arthropod-borne Virus Information Exchange appears a month later than scheduled. The following account of St. Louis encephalitis investigations which have occupied attention and resources of the CDC Arbovirus Unit almost exclusively for the past two months is primarily responsible for the delay. Editorial attention, preoccupation of typewriters with urgent reports, and forms demanded and remarkably supplied by the printshop have all displaced priority for this Infoexchange.

The deadline for the next exchange is December 15--before Christmas--so that we can make up for our postponement. It is understood that scientific productivity and research in other parts of the world did not cease just because the United States had an unusual year for arbovirus encephalitis.

SPECIAL REPORT FROM THE TEXAS STATE DEPARTMENT OF HEALTH VIRUS LABORATORY, AUSTIN, TEXAS; THE UNIVERSITY OF ILLINOIS CENTER FOR ZOONOSES RESEARCH, URBANA, ILLINOIS; THE NEW JERSEY STATE HEALTH DEPARTMENT PUBLIC HEALTH LABORATORIES, TRENTON, NEW JERSEY; AND THE COMMUNICABLE DISEASE CENTER ARBOVIRUS UNIT, ATLANTA, GEORGIA

The CDC Arbovirus Unit laboratory and field staff joined with personnel of Texas, Louisiana, Missouri, Tennessee, Illinois, Indiana, Kentucky, and New Jersey, to monitor and investigate what appears to be the most extensive dispersion of St. Louis encephalitis virus into human inhabitants on record in the United States. By far the largest number of human cases occurred in Houston, Texas, where the nature of the disease, which began early in July, was not recognized until the third week of August, when the City Health Officer, Dr. C.A. Pigford, moved by finding an unusual number of death certificates describing acute central nervous system disease in the elderly, submitted four acute-convalescent serum pairs from the Ben Taub Hospital for Dr. J.V. Irons, Director of the State Laboratory in Austin, where rise in titer of HI and CF antibodies for St. Louis encephalitis (SLE) virus was reported.

On request of the City of Houston and Dr. J.E. Peavy, Texas State Health Officer, on August 20, a CDC team went into operation the following day, with headquarters in the City Public Health Laboratory provided by Director Reuben Wende, and aided by the considerable personnel assistance of Dr. John R. Hall's immunization program staff. At the initiation of efforts to define the nature and extent of the epidemic, it appeared that most of the cases had entered Ben Taub Hospital. The accumulated specimens from these cases, submitted to Baylor School of Medicine Virus Laboratories, where they were examined for an enterovirus etiology, were on hand for tests with arbovirus antigens. In order to retrospectively obtain further etiological information on suspected cases in that area of the city, and to further define a distribution pattern which did not fit previous concepts of mosquito-dispersed SLE virus, antigens, other reagents, equipment, and technologist (Mr. Leo Chester) support were provided to the Baylor laboratory. Canvassing of hospitals and physicians for past and current cases reshaped the

geographical distribution to a more random distribution and stimulated submission of serological specimens.

The primary functions of the laboratory program initiated in Houston were to: 1) solicit and obtain acute and convalescent specimens for laboratory examination; 2) document the specimens according to case; 3) direct the specimens toward most expeditious examination in one of the three laboratories involved; 4) receive by telephone preliminary results for recording on the master record and interpretation; 5) prepare Xerox copies of the master form sheet for information of the epidemiologists, city health officials, and a report to the physician, often within 72 hours of submission of the specimen.

The utilization of the HI test for SLE was worked out along with the serial reporting mechanism during the 1962 SLE epidemic in Florida. It was learned there that SLE HI antibodies appear as soon as three days after onset of illness and in a population not generally exposed previously to Group B arbovirus infection, a presumptive diagnosis of SLE infection can be made early in the course of the disease. This served two useful purposes: 1) shaped early definition of the disease problem; and 2) attracted submission of specimens on suspected cases from private physicians seeking diagnostic clues to their patients' illness. This, of course, resulted in a more comprehensive assessment of the epidemic earlier in its ultimate course.

About ninety per cent of the presumptive diagnoses prove out on subsequent sera. About half of the reported cases were quickly put in the presumptive category. A shift to confirmed on the basis of rise in titer in convalescent sera is now occurring; so, of the more than 700 reported cases, it is expected that over 300 will prove valid--which amounts to one of the biggest SLE epidemics on record (Table I). There were 32 reported deaths, all but one over the age of 50. The epidemic onset and progress are illustrated in the series of histograms in Figure 1, issued by the CNS Disease Surveillance Unit of CDC.

Simultaneous with the epidemiological and laboratory investigation of cases shared between the three laboratories, an entomological team, supervised by Dr. Daniel Sudia, commenced work on August 22. Initial light-trapping efforts proved ineffective and sucking tube collections retrieved

Culex quinquefasciatus from culverts, chicken houses, and other peridomestic resting sites. To date, 38,278 mosquitoes have been processed. The last of 11 isolations of SLE virus from Houston mosquitoes was from collections made September 10. Actual number and source of isolations from mosquitoes appear in Table II. It indicates widespread dispersion and activity of SLE virus infected mosquitoes in the Houston epidemic situation.

Dr. Rex Lord initiated mist net bird collections, and animal trapping to obtain data on the possible vertebrate species involved was also initiated on August 22. Results of isolation attempts appear in Table III, and implied incidence of infection by HI screening appears in Table IV. With up to 25% of certain species possessing antibodies and virus isolations from peridomestic resident birds, the blue jay and mockingbird, it appears that the wild birds played a significant role in circulating the virus and supplying it to infect Culex quinquefasciatus mosquitoes, which appeared to be abundant because of the extensive amount of contaminated standing or slow-flowing water.

The vertebrate virus isolation, characterization, and identification, as well as serological studies, were under supervision of Dr. Philip Coleman. In attempting to convey an impression of the size of the investigative effort, there were times during the initial two weeks when as many as 25 CDC and related personnel were occupied in Houston, working with even more local medical and other professional personnel.

Although the first human case of encephalitis due to SLE in Illinois was recognized in McLeansboro in the southeast region of the state on August 5, it was not until the end of the month that an outbreak which eventually amounted to 19 suspect cases, 12 serologically proven, came under the scrutiny of Dr. R.H. Kokernot of the Illinois Center for Zoonoses Research. A histogram showing the cases by week of onset is presented in Figure I, the first known case having an onset during the week ending July 18.

Under the direction of Dr. Kokernot, intensive collections were undertaken of mosquitoes, bird bloods, and human survey sera. As of this date, over 1500 mosquitoes, representing 55 pools, have been processed, resulting in 30 possible isolates, one of which has been confirmed as SLE. This isolate is from a pool of Culex pipiens collected on September 4, 1964 (Table III).

In addition, tissues from over one hundred birds have been inoculated, resulting in the isolation of SLE virus from two house sparrows and a catbird collected in the McLeansboro area (Table III). As in Houston, there is evidence by HI test of a considerable number of birds collected having antibodies to SLE (Table V).

Dr. Norman Rose, Chief, Bureau of Epidemiology, Illinois Department of Public Health, has also reported eleven cases of serologically confirmed SLE from the Edwardsville-Alton area in Madison County in the western part of the state.

During the first week of September, Mr. Cliff Todd, Epidemiologist with the State Health Department, requested assistance from the CDC to investigate an outbreak of encephalitis in Boyle County (Danville), Kentucky, located in the central part of the state. Specimens from human cases collected by Mr. Todd and an EIS Officer of CDC were processed at CDC and results to date are summarized in Table II. Of the 36 cases on whom specimens have been received, 13 show presumptive or confirmed serological evidence of recent SLE infection. Once again referring to Figure I, the first case appeared during the end of the first week in August, the peak during the first week of September, and last suspect case on September 22.

Simultaneous investigation of the mosquito vector by a CDC team resulted in the collection of close to 5000 mosquitoes. From those processed to date, there have been one confirmed and two probable isolations of SLE virus (Table III) from Culex pipiens mosquitoes.

Mr. Herbert Maxfield, using mist nets, collected serum from 259 birds. These specimens have not been tested at this time.

The scope of the dispersion of SLE virus into this area of the country was further widened by the finding of another focus of encephalitis in Evansville, Indiana, in the southwestern part of the state, across the Wabash River from McLeansboro. Specimens submitted to CDC by Dr. Josephine Van Fleet have shown eight cases of presumptive or confirmed SLE infection in this area, the dates of onset ranging from August 22 to September 4.

Over 5000 mosquitoes for virus isolation were collected by Dr. Verne Newhouse from the Evansville area during the end of September; these are being processed at this time at CDC.

An additional focus of SLE activity became apparent in Camden County, New Jersey, during the middle of September. Dr. Martin Goldfield reports 88 suspect cases to date, 61 of which have been serologically confirmed. A histogram is shown in Figure 1, and further available information on cases in Table II. This is the first known occurrence of encephalitis due to SLE in this area of the country.

Collection of over 15,000 mosquitoes was undertaken in the Camden County area. No confirmed isolates are reported at this early date.

Just west of New Jersey in Philadelphia, Pennsylvania, there are eight suspect cases of encephalitis, one confirmed as SLE.

Evidence of infection with SLE has also been obtained from Memphis, Tennessee, with one confirmed clinical case, and SLE was isolated from two pools of C. pipiens collected in Memphis during the second week of September (Table III).

Figure I

HISTOGRAM OF HUMAN CASES OF ENCEPHALITIS BY WEEK OF ONSET FOR HOUSTON, TEXAS, ILLINOIS, KENTUCKY, AND NEW JERSEY

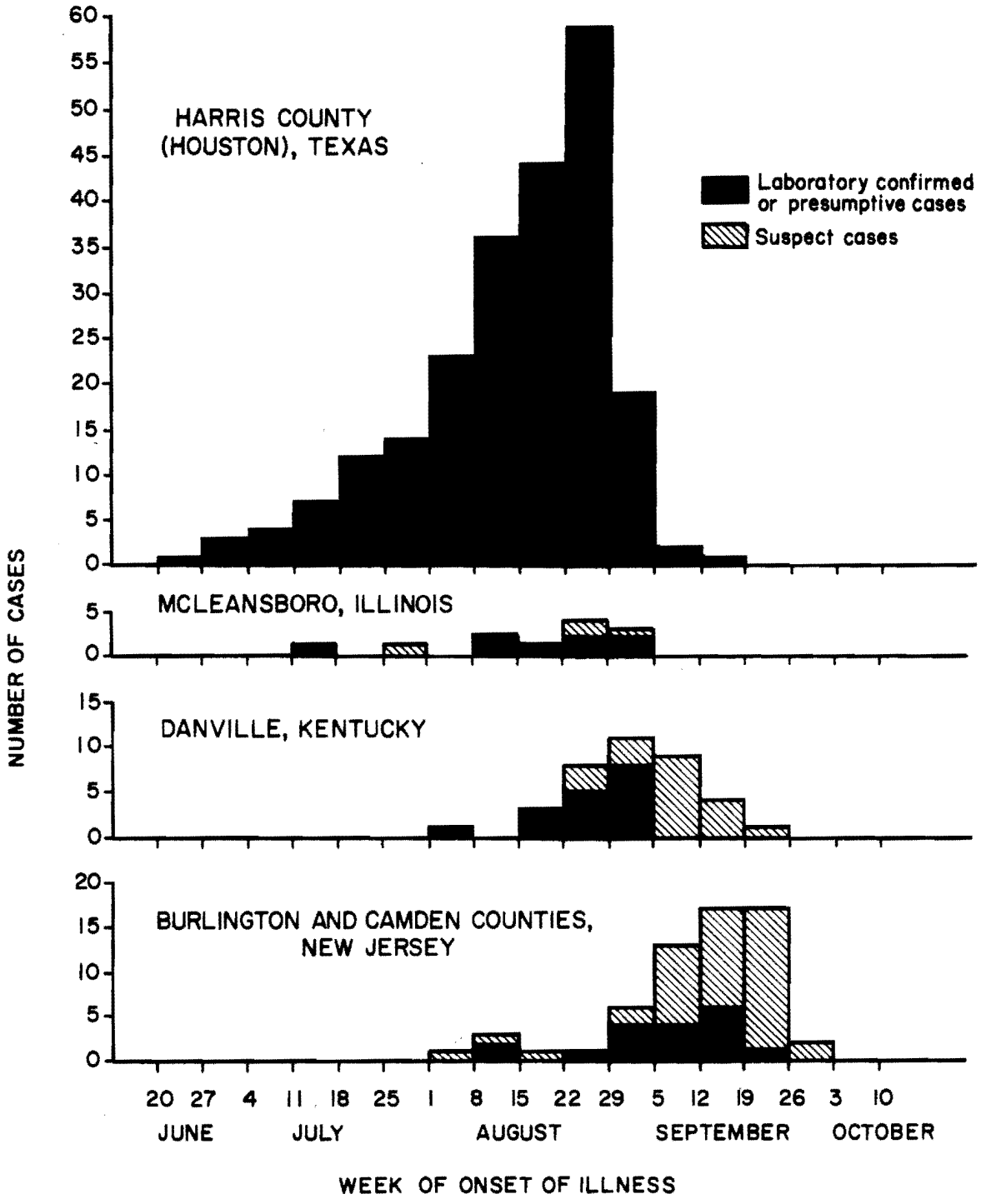


Table I

STATUS OF LABORATORY ANALYSIS OF SUSPECT HUMAN CASES IN HOUSTON, TEXAS, BY SEROLOGICAL TESTING TO SLE, THROUGH 10 OCTOBER 1964

AGE	TOTAL SUSPECT CASES	SPECIMENS RECEIVED	NEGATIVE	INCON-CLUSIVE	CONFIRMED OR PRESUMPTIVE	% CONFIRMED OR PRESUMTIVE OF SPECIMENS RECEIVED	ESTIMATED ACTUAL CASES
0-9	104	75	17	32	26	35%	36
10-19	101	79	10	45	24	30%	30
20-29	141	115	11	78	26	22%	31
30-39	92	79	9	41	29	37%	34
40-49	70	54	6	25	23	43%	30
50-59	70	50	4	21	25	50%	35
60-69	63	50	3	11	36	72%	45
70-79	44	34	0	10	24	70%	31
80+	22	15	0	1	14	93%	20
	707	551	60	264	227	41%	290

Table II

STATUS OF LABORATORY ANALYSIS OF SUSPECT HUMAN CASES IN DANVILLE, KENTUCKY, MCLEANSBORO, ILLINOIS, AND CAMDEN, NEW JERSEY, BY SEROLOGICAL TESTING TO SLE, THROUGH 10 OCTOBER, 1964

	SUSPECT CASES	SPECIMENS RECEIVED	NEGATIVE	INCON-CLUSIVE	PRESUMPTIVE OR CONFIRMED	% PRESUMPTIVE OR CONFIRMED OF SPECIMENS RECEIVED	ESTIMATED ACTUAL CASES
McLeansboro, Ill.	19	15	1	5	9	60%	11
Danville, Ky.	42	36	4	19	13	35%	15
Camden, N.J.	88	88	0	17	61	70%	61
Indiana	20	20	4	8	8	40%	8

Table III

STATUS OF LABORATORY EXAMINATION OF MOSQUITOES, WILD BIRDS, AND HUMAN AUTOPSY MATERIAL FOR ISOLATION OF SLE VIRUS IN TEXAS, TENNESSEE, ILLINOIS, INDIANA, KENTUCKY, AND NEW JERSEY (AS OF 7 OCTOBER 1964)

		NUMBER		ISOLATIONS		
	LOCATION	COLLECTED	INOCULATED	NO.	SPECIES	DATE COLLECTED
Mosquitoes	Houston	58,831	35,278	9	C. quinquefasciatus	8/24-28/64
				1	C. quinquefasciatus	9/10/64
				1	A. quadramaculatus	8/27/64
	McLeansboro	2,500	1,500 (55 pools)	30 pools suspect isolates		
				1	C. pipiens	9/4/64
	Danville	4,724	3,745	1	C. pipiens	9/12/64
				2	" (probable)	9/13,23/64
	Camden	15,000	*	*		
	Memphis	580	580	2	C. quinquefasciatus	9/8-11/64
	Evansville	5,200	*	*		
Wild birds	Houston	1,034	550	1	Blue Jay	8/24/64
				1	Mockingbird	8/27/64
	McLeansboro	171	125	2	House Sparrow	9/2-12/64
				1	Catbird	9/2-12/64
	Danville	259	*	*		
Human	Houston	7 (autopsy material)	7	0		
	Danville	0	0	0		
	Camden	0	0	0		

*Results of isolation attempts not available as of this date.

Table IV

STATUS OF EXAMINATION OF WILD BIRD SERA FOR INCIDENCE OF HI ANTIBODIES TO SLE IN HOUSTON, TEXAS (AS OF 25 SEPTEMBER 1964)

SPECIES	DATES OF COLLECTION								TOTALS		% POS.
	8/24-9/1		9/2-9/9		9/10-9/16		9/17-9/21				
	TOTAL	POS.	TOTAL	POS.	TOTAL	POS.	TOTAL	POS.	COLL.	POS.	
Mockingbird	46	8	31	5	7	-	10	2	94	15	1
Cardinal	25	2	9	1	7	-	11	1	52	4	7.5
Tufted Titmouse	9	3							9	3	
Blue Jay	65	14	31	8	17	6	26	15	140	43	32
Unidentified flycatcher	14		35	5	17	1	9		75	6	8
Carolina wren	5	1	4		2		2		14	1	
Screech Owl	5	2	2						7	2	
Great-crested flycatcher	6	1	4		1		2	1	13	2	
Redbellied woodpecker	7	1	2	1			1		10	2	
Common grackle	12	1	1		19		23	3	55	4	7
Robin	3		9	2					12	2	16
House sparrow	22	4	66	20			33	7	121	31	26
Baltimore oriole			12	4	20		3		35	4	11
Boat-tailed grackle					3	1	7	1	10	2	
Pigeons	100	18			100	21			200	39	19.5
Other species (30)	19	4	26	0	23	0	14	0	85	4	
Grand Total	338	59	232	46	216	29	141	30	932	167	17.9

Table V

STATUS OF EXAMINATION OF WILD BIRD AND DOMESTIC FOWL SERA FOR INCIDENCE OF HI ANTIBODIES TO SLE IN MCLEANSBORO, ILLINOIS (AS OF 25 SEPTEMBER 1964)

Species	Total Collected	HI Positive to SLE or MVE	Per Cent Positive
Mourning Dove	14	0	0
Common grackel	11	0	0
Blue Jay	15	8	53
House sparrow	58	24	41
Chimney swift	101	11	11
Pigeon	37	28	76
Starling	10	1	10
13 other species	25	9	36
	271	83	30