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ARBOVIRAL DISEASE SECTION
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ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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REPORT ON THE CATALOGUE OF THE ARTHROPOD-BORNE VIRUSES OF THE WORLD, MARCH, 1965 BY R.M. TAYLOR, CHAIRMAN, SUBCOMMITTEE ON ARBOVIRUS INFORMATION EXCHANGE

The second revision of the Catalogue was issued in January of this year. Thanks to the collaboration of the contributors, it included the re-registration of all of the previously registered viruses, with the exception of seven which were voluntarily dropped, five because they were not deemed sufficiently different from other registered or published viruses, and two because of insufficient evidence that they belonged to the arbovirus category. This issue of the Catalogue contains cards on 161 viruses, 134 of which were previously registered, and 27 represent new additions during the past six months. As far as we are aware, the Catalogue includes all of the arboviruses that had been published at the time of issue (January, 1965) and an additional 45 which had not been published. As registration is voluntary, there are no doubt a number of unpublished viruses that have not been registered, probably a dozen or more, either because of lack of sufficient information to warrant registration, or for lack of interest in the exchange of restricted information with other investigators.

Two hundred copies of the revised Catalogue have been assembled. In addition to 89 previous recipients, 15 have been added, and these have received not only the Catalogue in a loose-leaf binder, but also a full set of previously issued abstracts and current information slips approximating 2,800, coded and collated with index cards for filing.

So far, there has not been an opportunity to adequately compile and analyze the data that may be extracted from this Catalogue. It will be done later and distributed in a future issue of the Information Exchange. However, a graph and a few tables are included in this report which relate to the dramatic increase in the number of arboviruses discovered since 1950. In addition, some information is given on disease produced by these viruses in nature and in the laboratory.

The analyses which follow include data from 160 viruses. One, the "Hanzalova" strain (Catalogue No. 153) of the tick-borne encephalitis (TBE) complex, has been omitted as it is probably the same virus as the TBE "Hyper" strain

(Catalogue No. 154). The attached graph showing the year of isolation of these 160 viruses is somewhat misleading because it does not, for lack of space, show the intervals between isolations prior to 1950, but it does illustrate the precipitous rise in the number of isolations during the 1950's. Although the number per year appears to have tapered off since 1955, the present rate indicates that there may be as many or more isolates during the present decade than during the preceding one.

An attempt to present and gauge some of the factors which may have contributed to this increase in the discovery of "new" arboviruses is shown in Table 1. This table gives information on the source, evidence of illness, and nature of study at progressive time intervals. That is: was the virus isolated from man, lower vertebrates, sentinel animals, or arthropods? Was there any evidence of illness, if isolated from man or lower animals, and what was the nature of the study that led to isolation? In this case, was it to investigate the same disease that was symptomatically manifest, or was the isolation made during the study of the epidemiology of another disease, or was it made during a general survey for arboviruses? These classifications are arbitrary and decisions were not always easy to make, but it is believed that for the purpose of this analysis, they are sufficiently correct.

It will be noted that all of the 15 isolations prior to 1940 were made from man or other vertebrates (domestic) manifestly ill and that on 13 occasions the purpose was to study the same disease that was symptomatically manifested. On two occasions, the isolations were made while investigating the epidemiology of another disease--in these instances, yellow fever.

During the next decade (1940-49) ten of the 18 isolates were made from arthropods and 1 from a sentinel monkey. The remaining seven were from man during illness. Yet, as in the preceding decade, the object of the studies was to investigate either the same disease that was symptomatically manifest, or another known disease--mainly yellow fever.

Table 1

Date, Source and Circumstances (Nature of Study) of Isolation of 160 Viruses Registered in Catalogue (January 1965)

Date of Isolation		Source				Evidence of Illness		Nature of Study		
Year	No.	Man	Other Vertebrates	Sentinel Animal	Arthropods	Man	Other Vertebrates	Same Disease	Another Disease	General Survey
1905	1		1				1	1		
1910	1		1				1	1		
1925	1		1				1	1		
1927	1	1				1		1		
1929	1		1				1	1		
Before 1930	5	1	4			1	4	5		
Percent	3%	20%	80%			20%	80%	100%		
1930	2		2				2	2		
1932	1		1				1	1		
1933	2	1	1			1	1	2		
1935	1	1				1		1		
1937	3	3				3		1	2	
1938	1		1				1	1		
1930-1939	10	5	5			5	5	8	2	
Percent	6%	50%	50%			50%	50%	80%	20%	
1940	3				3				3	
1942	1				1				1	
1943	5	2			3	2		2	3	
1944	4	3			1	3		3	1	
1947	3	1		1	1	1		1	2	
1948	2	1			1	1		1	1	
1940-1949	18	7		1	10	7		7	11	
Percent	11%	39%		6%	56%	39%		39%	61%	
1951	2	2				2		2		
1952	2				2					2
1953	3	2			1	2		2	1	
1954	8	1	2	3	2				2	6
1955	24	3	2	4	15	3	2	5		19
1956	12	2	2	2	6	1		1	2	9
1957	12	3	3	1	5	3	1	4	1	7
1958	8	2	3		3	2	1	3		5
1959	15	1	2	2	10	1	1	2		13
1950-1959	86	16	14	12	44	14	5	19	6	61
Percent	54%	19%	16%	14%	51%	16%	6%	22%	7%	71%
1960	17	2	3		12	2		2		15
1961	10		2	2	6					10
1962	11	1	7	1	2	1		1	1	9
1963	3	1			2	1		1		2
1960-1963	41	4	12	3	22	4		4	1	36
Percent	26%	10%	29%	7%	54%	10%		10%	2%	88%
Grand Total	160	33	35	16	76	31	14	43	20	97
Percent		21%	22%	10%	47%	19%	9%	27%	13%	60%

In the 1950's, a marked change began in the number and sources of the isolates, and in the nature of the studies. The total number of isolates greatly increased (86), but those from man proportionately diminished, and those from sick domestic vertebrates virtually ceased. The general survey introduced during this decade had, as its object, the search for arboviruses wherever they were likely to be found, and with its advent, isolations from arthropods, small wild mammals and birds, and sentinel animals, began to dominate the picture. This trend has continued. The breakdown of "Other Vertebrates" in Table 1 is given in Table 2.

Table 2

Other Vertebrates Than Man From Which Initial Isolation of Viruses Was Made (January, 1965)

Domestic		11
Sheep	5	
Horse	4	
Cow	1	
Turkey	1	
Wild		24
Bats	7	
Rodents	5	
Lizards	3	
Birds	2	
Marsupials	2	
Monkeys	2	
Deer	1	
Rabbit	1	
Armadillo	1	
Sentinel Animals		16
Monkeys	11	
Infant mice	5	
Total		51

The striking increase in the number of isolates in the mid-1950's is in large part attributable to undertaking general surveys for arboviruses in Africa, India, and South America with the aid of the Rockefeller Foundation, and under other auspices, in Malaysia. Such general surveys pursued elsewhere, i.e., Australasia and the Middle East, have also contributed to the growing list of arboviruses.

Another factor which was indubitably of great importance in the increase of arbovirus isolations was the use of infant mice as a routine laboratory procedure. The first isolation by this method was Wyeomyia virus by Roca-Garcia in 1940 in connection with yellow fever investigations in Colombia, and the second was Trivittatus virus by Eklund in 1948 in the course of investigating WEE. It was not, however, until the early fifties that it became a routine procedure in general surveys as exemplified by the isolation of Sindbis virus in Egypt in 1952. The methods that have been used successfully in the laboratory for the initial isolation of arboviruses are listed in Table 3.

Table 3

Methods of Initial Isolation (Discovery) of Virus Arranged According to Frequency

(160 Viruses, January 1965)

Method	No.
Infant or suckling mice	115
Weanling or adult mice	31
Man	4
Monkey	4
Sheep	4
Guinea pig	4
Embryonated egg	3
Hamster - adult	1
Hamster - infant	1
Horse	1
Deer	1
Tissue Culture	1
Rabbit	1
Total	171

Isolations by more than one method 11; i.e., infant and adult mice 8, infant mice and embryonated egg 1, adult mice and monkey 1, and guinea pig and rabbit 1.

Table 4 shows the chronological frequency of initial isolations by suckling mice, by adult mice, and by all other methods. It is evident that isolations in infant mice synchronize with the increase in the total number of isolates. Available data do not permit accurate determination of the number of isolations that would have been missed if weanling, instead of infant, mice had been used in these general surveys--a guess would be from 50 to 75 per cent.

Table 4

Chronological Frequency of Isolations by Different Methods

	Total Isolations	Mice Suckling No.	Mice Suckling %	Mice Adult No.	Mice Adult %	All Other No.	All Other %
Before 1930	5	0	0	0	0	5	100
1930-1939	10	0		6	50	6	50
1940-1949	18	1	6	10	56	7	19
1950-1959	86	75	80	13	14	6*	6
1960-1963	41	39	93	2	5	1*	2
Total	160	115	67	31	18	25	15

*Where sum of numbers under methods exceed the total isolations for the period it is due to isolations by more than one method (see note following above table).

It appears, therefore, that the two most important factors in augmenting the discovery of arboviruses were the institution of general surveys, especially in the tropics, and the routine use of infant mice for virus isolation. Also, utilization of sentinel animals as a means of trapping the virus in the field should be mentioned as a significant contributing factor.

This background of information and its implications have been presented because the data on the virus source and methods employed in obtaining the initial isolate are not obtainable by the key-sort system and, it is hoped, may be of some interest to those now actively engaged in the discovery of "new" viruses.

In a follow-up and more complete analysis, the relation between subsequent human infection and the source of the initial isolate will be presented. The present evidence indicates that viruses initially isolated from monkeys, either wild or sentinel, or from domestic quadrupeds, are more likely to infect and cause disease in man than those isolated from any other source, except, of course, man himself.

The revised questionnaire for this issue of the Catalogue requested information pertaining to human disease in nature and in the laboratory. Table 5 shows analyses of the returns on virus isolations from man in nature, manifest disease in nature, and overt laboratory infections, tabulated according to the period of initial isolation of the virus. As would be anticipated, the frequency of these occurrences tends to increase with lapse of time since the virus was initially discovered. There is almost a perfect correlation between viremia in man and manifest symptoms. This is to be expected, since human bloods for attempted virus isolation are usually collected during manifest illness. As will be noted, the overall percentage of detected viremia in man is 36% among the 160 viruses included in the analysis, and manifest disease both "reported" and "significant" totals 38%.

Table 5

Evidence of Human Infection by: (a) Virus Isolation in Nature; (b) Disease in Nature, (R) Reported, (S) Significant; (c) Overt Laboratory Infection, (R) Reported, (S) Significant Tabulated According to Period of Initial Isolation of Virus.

	Total No.	Virus Isolation in Nature		Disease in Nature (R)		Disease in Nature (S)		Overt Laboratory Infection (R)		Overt Laboratory Infection (S)	
		No.	%	No.	%	No.	%	No.	%	No.	%
Before 1930	5	3	60	1	20	2	40	1	20	3	60
1930-1939	10	9	90	1	10	8	80	4	40	3	30
1940-1949	18	11	61	5	28	6	31	5	28	2	11
1950-1959	86	30	35	17	20	14	16	8	9	3	3
1960-1963	41	5	12	4	10	2	5	2	5	2	5
Totals	160	58	36	28	18	32	20	20	13	13	8

Note: In addition to the overt laboratory infections, subclinical infections have been reported with EEE, Ilheus and Semliki.

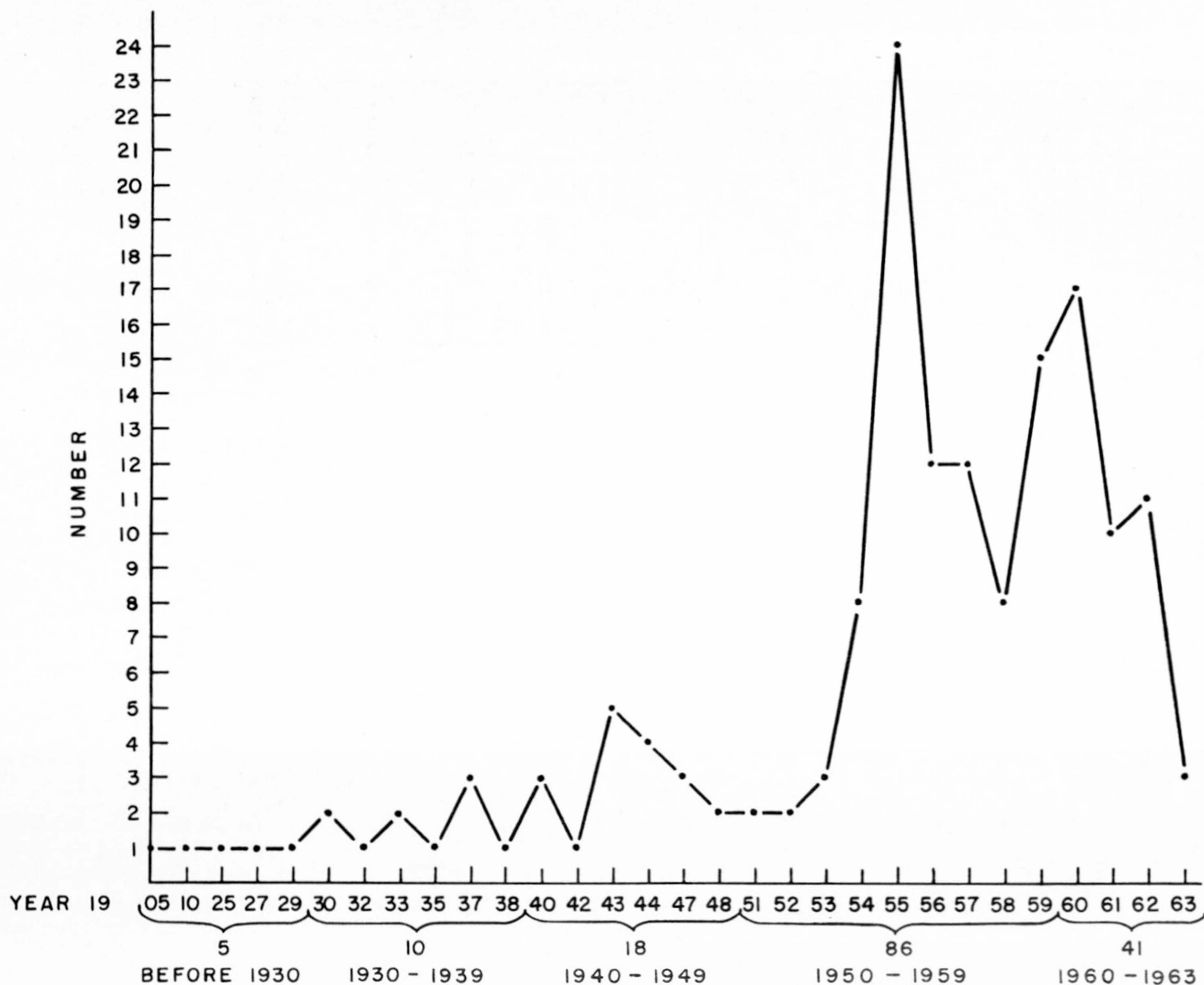
Pending the report of the Subcommittee on Laboratory Infections, attention is directed to a total of 21% (13% reported and 8% significant) overt laboratory infections recorded on the Catalogue cards. In order that laboratory personnel handling arboviruses may be forewarned, a list of those viruses that have produced accidental overt infection in the laboratory is included in this report, Table 6.

Table 6

List of Viruses That Have Produced Overt Accidental Laboratory Infection. March, 1965

Reported (R)	Significant (S)
Bunyamwera	Absettarov
Chikungunya	Colorado Tick Fever
Dengue I	Kemerovo
Dengue II	Hyper
Germiston	KFD
JBE	Louping Ill
Junin	Omsk HF
Kunjin (MRM 16)	Piry
Mucambo	Rift Valley Fever
Nairobi Sheep Disease	RSSE
Negishi	VEE
Oropouche	VSV - Indiana
Rio Bravo (M 64)	Yellow Fever (Asibi)
SLE	
Spondweni	
Machupo	
WEE	
Wesselsbron	
WEST Nile (B 956)	
Zika	

Reported (R) indicates infrequent or mild.
Significant (S) indicates frequent or severe.


YEAR OF ISOLATION OF 160 VIRUSES REGISTERED IN THE CATALOGUE
JAN. 1965
NUMBER
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
YEAR 19
05 10 25 27 29 30 32 33 35 37 38 40 42 43 44 47 48 51 52 53 54 55 56 57 58 59 60 61 62 63
5
BEFORE 1930
10
1930 - 1939
18
1940 - 1949
86
1950 - 1959
41
1960 - 1963

REPORT FROM DR. A.C. SAENZ WORLD HEALTH ORGANIZATION, GENEVA, SWITZERLAND

From 19-26 October, 1964, the WHO Seminar on Mosquito-Borne Hemorrhagic Fevers in Southeast Asia and the Western Pacific took place in Bangkok. Twenty-seven participants and more than 80 observers attended this seminar. Drs. Halstead, Hammon, Lim Kok Ann, Tunchinda, and Rudnick acted as WHO consultants during the seminar. Copies of the report of the seminar are available on request.

The following "SUGGESTED DEFINITION AND NOMENCLATURE FOR DISEASES SUSPECTED TO BE OF DENGUE OR CHIKUNGUNYA VIRUS ETIOLOGY" was included as an annex to the report:

> "It must be recalled that mosquito-borne fevers are multiple in etiology. In addition, many fevers of a mild and relatively undifferentiated nature are caused by enteroviruses and by many other infectious agents. The physician seeing such a patient during an epidemic which is believed to be caused by an arbovirus, seldom can differentiate clinically in any individual patient in respect to etiology and should be content to call it a fever of known origin or an undifferentiated fever. Presence or absence of a rash adds little of diagnostic significance. When etiology is established and the clinical syndrome is undifferentiated, diagnosis should remain as an undifferentiated fever of the specified etiology, e.g., due to dengue virus type 3 or due to chikungunya virus.
>
> A fairly well established clinical syndrome of dengue-like disease has been recognized and fairly well accepted for many years. Certain cases in some countries and in certain age groups (usually adults) fit easily into this category. Etiological diagnosis if available should be added, e.g., dengue fever syndrome due to chikungunya virus.
>
> If the disease is associated with relatively severe hemorrhagic manifestations and/or shock and is in other ways compatible with the more severe grades of what has been called Philippine,

"Thai, or Singapore hemorrhagic fever, the term hemorrhagic fever[1] would be indicated. Again, etiology should be added if available.

On the basis of the above, the following is suggested:

I. Undifferentiated fever.

1. Etiology unknown.

2. Etiology stated.

II. Dengue fever syndrome. This should include febrile disease characterized by myalgia and/or arthralgia and leucopenia, with or without rash or lymphadenopathy, but including many of the following: biphasic fever, severe headache, pain on moving the eyes, positive tourniquet test[2], and a few spontaneous petechiae.

1. Etiology unknown.

2. Etiology stated.

III. Hemorrhagic fever[3]. This includes fever, usually without prominent myalgia or arthralgia, positive tourniquet test, leucopenia or rash present or absent, but usually with several of the following:

---

[1]In areas where tick-borne or mite-borne hemorrhagic fevers also exist, it might be wise to use the longer title mosquito-borne hemorrhagic fever if the other types can be reasonably well ruled out by epidemiological observations.

[2]The tourniquet test should be employed in a standardized manner (e.g., Rampell-Leede's test). This should be done with a blood pressure cuff and not with a tourniquet.

[3]To be considered in differential diagnosis are: thrombocytopenic purpura, haemophilias of genetic origin, menigococcemia, scarlet fever, hemorrhagic measles, rubella, enterovirus infections with rash, rickettsial spotted fevers such as scrub typhus, leptospirosis, etc.

"extensive spontaneous petechiae, purpura, acehymoses, epixtaxis, hematomesis, melaena, thrombocytopenia, prolonged bleeding time and maturation arrest of megakaryocytes. This is further subdivided into:

A. Without shock.

1. Etiology unknown.

2. Etiology stated.

B. With shock. The pulse pressure is 20 mm of Hg or less, or systolic and diastolic pressures unobtainable, with collapse of the patient. Shock may occur without the hemorrhagic manifestations described above, but with most of the following associated with serious disturbance of the hemostatic mechanism as essential criteria if diagnosed hemorrhagic fever: positive tourniquet test, thrombocytopenia, prolonged bleeding time, and maturation arrest of the megakaryocytes.

1. Etiology unknown.

2. Etiology stated."

REPORT FROM DRS. SCOTT HALSTEAD AND CHARAS YAMARAT, VIRUS DEPARTMENT, U.S. ARMY-SEATO MEDICAL RESEARCH LABORATORY AND SCHOOL OF PUBLIC HEALTH, BANGKOK, THAILAND

1. WHO Seminar on Mosquito-Borne Hemorrhagic Fevers in the Western Pacific and Southeast Asia Regions, Bangkok, October 19-26, 1964.

As perhaps is more extensively reported elsewhere in this edition, a highly informational seminar on the above subject was held in Bangkok in October, 1964. Representatives from 15 countries in the two WHO regions, including five countries with experience with virologically documented mosquito-borne hemorrhagic fever, attended the meeting. Some of the observations and recommendations made at the seminar were:

a. In 1964, hemorrhagic fever outbreaks due to dengue viruses have occurred in the Philippines, South Vietnam, Malaysia, Thailand, and India (small outbreak).

b. Dengue virus types 1 and 4 have been isolated in Phnom Penh, Cambodia, where no hemorrhagic fever is reported. Dengue viruses 1, 2, and 4 have been the cause of human infection over a period of 4 years in Vellore, (South) India without coincident occurrence of hemorrhagic fever.

c. Chikungunya virus has been the cause of human disease in Cambodia, Thailand, Burma, and India. The 1964 outbreak in India is reportedly very large. In addition, chikungunya antibodies have been found in residents of South Vietnam and East Pakistan.

d. In Thailand, chikungunya virus is <u>not</u> a cause of the severe hemorrhagic fever syndrome.

e. Severe hemorrhagic fever has been more carefully defined than heretofore possible. Physiologic studies of virologically confirmed dengue infections in Thailand have shown that most of the features of the disease can be explained by the shock syndrome. Fluid and protein leaks from damaged capillaries result in decreased blood volume, increased hematocrit, and tissue hypoxia. Without therapeutic intervention, many patients die apparently with terminal hyperkalemia. Gastrointestinal hemorrhage appears to follow shock. Measured hemostatic abnormalities including thrombocytopenia were usually not severe enough to account for spontaneous bleeding. A treatment schedule employing only replacement protein and electrolyte solution has been tested with good results in 100 confirmed dengue hemorrhagic fever patients in Thailand. Results of recent studies question the usefulness of steroids and broad spectrum antibiotics.

f. Febrile disease with positive tourniquet test, thrombocytopenia, petechiae, or purpura with or without epistaxis, gastrointestinal hemorrhage, rash, hepatomegaly, myalgia, or arthralgia should be referred to as hemorrhagic fever, <u>without shock</u>, etiology to be specified.

g. It was recommended that non-specific febrile diseases characterized by thrombocytopenia, positive tourniquet test or sparse petechiae as their only hemorrhagic manifestations <u>not</u> be referred to as hemorrhagic fever, but as undifferentiated fever, etiology to be specified.

h. Dengue fever as a clinical diagnosis is to be reserved for the syndrome characterized by myalgia and/or arthralgia and leucopenia, with or without lymphadenopathy, rash, biphasic fever, thrombocytopenia, and positive tourniquet test. In South and Southeast Asia, this syndrome has been caused by dengue and chikungunya viruses.

i. Problems associated with dengue virus typing, the pathogenesis of hemorrhagic fever, recovery of etiologic agents from autopsy materials, the ecology of dengue and chikungunya viruses, and control of the vector mosquito were discussed. The need for immediate mosquito control in endemic areas and the danger of spread of disease to other _Aedes aegypti_ infected areas was stressed.

A comprehensive final report of the seminar was prepared. Inquiries for extra copies should be directed to Dr. Arturo Saenz, Virus Diseases, Division of Communicable Diseases, WHO Headquarters, Geneva.

2. _Thai hemorrhagic fever study ward_. The joint SEATO Medical Research Laboratory, Thai Army and Ministry of Public Health hemorrhagic fever study ward ceased operation in October. One hundred and fifty-one patients were admitted for treatment. Numerous biochemical, bacteriological, and virologic studies were made on all patients. From physiologic studies accomplished, a management regimen based upon microhematocrit determinations was developed. Scientific studies were under the direction of Captain Sanford Cohen, Walter Reed Army Institute of Research. Reports are being readied for publication.

3. _Hemorrhagic fever in Thailand, 1964_. Estimated total hemorrhagic fever admissions throughout Thailand in 1964 are 8-10,000 with an overall mortality rate of approximately 7%.

4. _Japanese encephalitis outbreak_. An outbreak of clinical Japanese encephalitis is reported for the first time from Thailand. Disease occurred on the northern portion of the Central Plain. Over one hundred cases were admitted to Pisanuloke Hospital. Total hospital admissions in the area are unknown. Cases at the Pisanuloke Hospital occurred with equal frequency in all age groups. This is consistent with our earlier observation that only 50% of 20-year-old Thai residents in the area had JE neutralizing antibody in 1962 (perhaps of dengue virus origin?).

REPORT FROM THE DIRECTOR, VIRUS RESEARCH CENTRE POONA, INDIA

Chikungunya virus in South India.

Towards the end of August, 1964, word was received from Madras that a "break-bone" fever was widely prevalent in the city. Investigations were immediately initiated. During the preliminary visit by the staff of the Virus Research Centre, Poona, (VRC) it was ascertained that cases with typical dengue characteristics were occurring all over the city. While no definite date of the commencement of the outbreak could be determined, it was found that the cases were already being noticed in the month of July and by the middle of August they had reached epidemic proportions. Clinical observations on the disease in Madras city were being made at the Stanley Medical College Hospital and at the Infectious Diseases Hospital, Madras. A similar illness was also prevalent in a few other cities in South India, particularly Pondicherry and Vellore. At the latter place, clinical investigations are in progress at the Christian Medical College Hospital.

The signs and symptoms of the disease are: There is sudden onset of fever accompanied by severe pain all over the body, particularly in the joints. The fever lasts for 3-4 days and comes down by crisis. In a proportion of cases, a second bout of fever of lesser severity occurs after a lapse of two days. A rash variously described as maculo-papular, morbilliform, etc. has been noticed in many cases in the later phases of illness. No frank hemorrhagic symptoms were apparently noticed in the earlier phases of the outbreak, but in September several cases with mild hemorrhagic phenomena have been observed. No deaths were reported in the earlier phases of the outbreak, but a few suspected to be due to the disease are stated to have occurred in late September.

No part of Madras city seems to have escaped and there have been cases in most households. Preliminary studies showed that *Aedes aegypti* was prevalent in the city.

Virus isolation: Three hundred fifty-four isolations of chikungunya virus have been made from August to December 1964. Two hundred fifty-eight of these were from sera of

patients and 96 from Aedes aegypti mosquitoes. Vellore being the headquarters of the field station, the number of patients examined there was much higher than in Madras city itself and therefore provided the bulk of the isolations (238 out of 258). Mosquito isolations were 48 from Vellore, 35 from Madras, and 15 from Pondicherry.

Serological studies: HI tests were carried out at the Poona laboratory on 145 pairs of sera collected in Madras from patients (acute and convalescent). Fifty-five and nine-tenths per cent of the pairs showed conversion to chikungunya virus only. Another 4.8% showed conversion both to chikungunya and Group B arboviruses (Table 1). Conversion or rise in antibody titre to Group B virus only occurred in 1.4% (total 6.2%). On the other hand, 84.1% of the acute sera already had HI antibodies to Group B viruses while only 19.3% to chikungunya. These data demonstrate that chikungunya virus was the one which was mainly associated with the febrile outbreak under study. Dengue viruses are known to be endemic in several cities in Madras State and could have played a minor role in the outbreak.

Eight hundred and fifty sera have been collected in December from a sample of non-febrile patients attending the outpatient departments of the major hospitals in Madras city, particularly from surgical, maternity, and gynaecological wards. These are expected to provide a fair sample of the cross section of the city to determine how the outbreak did affect the immunological status of the population. The outbreak appears to be on the wane from the middle of November.

Extensive serological surveys conducted in South India previously by the VRC had shown the prevalence of dengue in the entire region. The sera had been previously tested against Sindbis virus without any significant finding. They had not been tested against chikungunya, but in a very recent series of HI tests on the same sera, there seems to be some indication of a previous activity of chikungunya virus or a closely related virus in South India. Thirty-three of the 298 sera were positive in HI tests against chikungunya virus. All but four were above the age of 20 years and most of them above the age of 40 years. These findings are similar to those made in Calcutta and Jamshedpur in eastern India.

Summary of haemagglutination inhibition (HI) test results on paired sera from febrile cases in Madras City - August-November, 1964.

NOTE:- The sera were tested against the viruses of -

(A) Sindbis, chikungunya

(B) Japanese encephalitis, West Nile, dengue 1, dengue 2 and Kyasanur Forest disease.

	Infectious Diseases Hospital	Stanley Hospital	Total	Per cent
Total number of pairs with valid tests	31	114	145	100.0
(A) Conversion or rise in antibody titre*				
Chikungunya only	17	64	81	55.9
Group B arboviruses only	0	2	2	1.4
Both chikungunya and Group B	1	6	7	4.8
(B) Presence of antibodies in the acute serum				
Chikungunya only	0	1	1	0.7
Group B only	17	78	95	65.5
Both chikungunya and Group B	8	19	27	18.6

* In case of chikungunya the conversion was very clear, from negative to positive, in most cases. In cases of Group B arboviruses, however, there were more cases of rise in antibody titre.

Outbreak of dengue in Visakhapatnam, Andhra Pradesh, South India.

Early in September 1964, information was received that "hemorrhagic fever" cases were being admitted at the King George Hospital, Visakhapatnam. A team from the Virus Research Centre, Poona (VRC) proceeded to Visakhapatnam immediately. It was noticed that in one of the wards of the hospital, cases of a dengue-like illness were being admitted. The physician in charge had noticed mild hemorrhagic symptoms in 16 out of 86 cases he had observed. Pain in the joints was a feature in some cases. The inquiries also showed that a fever of this type was prevalent throughout the city. In the Medical College hostel itself, more than 25 per cent of the inmates had suffered.

A rapid inspection of some of the localities of the town where the cases had occurred showed the prevalence of *Aedes aegypti* though not on the same scale as in Madras. (Later a team from the National Institute of Communicable Diseases, Delhi, carried out a regular mosquito survey of the city).

The VRC team brought back to Poona acute and convalescent sera as well as sera from a number of contacts. The results of the tests done on them may be summarized as follows.

1) The hemagglutination inhibition tests on 156 sera did not show any activity of the chikungunya or Sindbis viruses.

2) There was serological conversion to Group B viruses in 23 out of 47 paired sera tested.

3) Fifteen out of 19 acute sera inoculated into *M. radiata* kidney epithelial cell (MKEC) cultures have yielded agents which, while not producing any CPE, interfere with the CPE of Polio 1 virus. Five of these strains have been identified as probably dengue 2 by virus neutralization in tissue culture. Immune sera against dengue 1, 2, 3, and 4 were used, but only dengue 2 sera could neutralize the polio I interfering effect of the agents.

4) From 14 pools containing 348 females of *Aedes aegypti* processed in MKTC, three agents, still to be identified, have been isolated.

The tentative conclusion of this study, still in progress, is that the outbreak was due to dengue viruses. It is interesting that indications of chikungunya virus activity have been totally absent.

Ecological Studies on Arboviruses at Manjri - A Special Study Area near Poona, India.

During the investigations on the epizootic of African horse sickness in 1960-61 and later on, an outbreak of rabies in horses occurring in a stud farm near Poona, HI antibodies to Chittoor virus (Bunyamwera Group) had been detected in a substantial proportion of horses indicating a possible activity of arboviruses in this locality eight miles from Poona. It had also been previously found that a person residing in a village close by had neutralizing antibodies to Kyasanur Forest disease virus. Sufficient information was also available on the arthropod fauna of this area. It was therefore thought that the area would provide good opportunities for a detailed scientific study of the ecology of arboviruses, one of the advantages being its close proximity to Poona. A study was initiated in March 1964.

The program of work included:

1) Weekly collection of arthropods including mosquitoes.

2) Trapping of small mammals and birds.

3) Periodical collection of blood from horses and other sentinel animals.

4) Processing of material for virus isolation and serological tests to determine the virus activity.

The work is still in preliminary stages but has already yielded some interesting results.

1) A virus identified as West Nile has been isolated from two pools (48 and 50) female *Culex fatigans*. Though there was serological evidence of West Nile infection in humans in this area, these are the first reports of the isolation of this virus in Western India. The characteristics of this virus are now being studied.

2) Chittoor virus has been isolated five times from mosquitoes: once from *Culex bitaeniorhynchus* (46), thrice from *Anopheles tessellatus* (50, 50, 38), and once from *Anopheles subpictus* (3).

3) An agent has been isolated from the lung of a dead horse (and possibly from a mosquito pool), which seems to belong to Psittacosis-Lymphogranuloma Venereum-Trachoma complex, or the Bedsonia group.

4) Serological studies in HI tests on large mammals have shown that:

a) Chittoor virus antibodies were found in 48 out of 177 horses of all ages, in 1 out of 17 sheep, in 36 out of 65 bovines, and in none of the 30 humans.

b) Antibodies to JE-WN group were found in 24 out of 60 humans, in 5 out of 177 horses, and in one out of 65 bovines.

c) Dengue antibodies were found in 6 out of 30 humans and in none of the animals.

d) Chikungunya antibodies were found in 1 out of 30 humans, in 6 out of 177 horses, and in 1 out of 17 sheep.

It has also been found that in foals, yearlings, and calves, the serological conversions to Chittoor and West Nile viruses take place during monsoon months.

5) The results of serological studies on small mammals have shown that:

a) None of the 17 *Tatera indica*, 26 *Rattus r. rufescens*, 7 *Suncus murinus*, 2 *Golunda ellioti*, 15 short-nosed fruit bats, and 6 fruit bats had antibodies to Chittoor virus.

b) HI antibodies to a Group B virus were found in 5 out of 26 house rats.

c) Chikungunya antibodies were found in 1 Indian Bush Rat and 1 House Rat.

6) An observation of considerable interest was that sera of a few bats (Rousettus leschenaulti) collected in this area neutralized KFD virus. Two out of 6 inhibited HA, and 3 out of 6 neutralized the virus in infant mice.

7) Serological tests on mist-netted birds showed that none of the 80 birds of 11 species had antibodies to Chittoor. Group B antibodies were found in three birds and chikungunya antibodies in one bird.

8) The small mammals collected have also been examined for their ectoparasites and a species of Rhipicephalus (tick) which appears to be a new one has been detected on Tatera indica.

Very preliminary as these results are, they have shown a high degree of arbovirus activity in this area, and it is hoped that the study will provide interesting data in regard to the natural history of such viruses.

REPORT FROM THE DEPARTMENT OF VIRUS DISEASES, WALTER REED ARMY INSTITUTE OF RESEARCH; AND THE PAKISTAN MEDICAL RESEARCH CENTER OF THE INSTITUTE OF INTERNATIONAL MEDICINE, UNIVERSITY OF MARYLAND

A severe epidemic of a dengue-like illness occurred in Dacca, East Pakistan, in the late summer of 1964. The illness was characterized by fever, myalgia, and headache. A transient rash was occasionally observed. No hemorrhagic manifestations were seen.

Twelve strains of viruses have been isolated from sera collected in early September 1964. The sera were obtained from Dacca University students on the first or second day of illness.

Virus isolations were made in primary grivet monkey tissue culture and in the BSC-1 line of continuous grivet monkey kidney tissue culture using the interference technique described by Halstead. One of these strains has been tested by neutralization test. It is neutralized by dengue antiserum and appears to be more closely related to type III than types I, II, or IV Attempts to propagate these strains in suckling mice have so far been unsuccessful.

(P.K. Russell, E.L. Buescher, F.R. McCrumb)

REPORT FROM DR. HERBERT C. BARNETT
INSTITUTE OF INTERNATIONAL MEDICINE
UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE, BALTIMORE;
AND THE PAKISTAN MEDICAL RESEARCH CENTER, LAHORE

Processing of mosquito material and identification of viral isolates from materials collected in 1962 was completed. Our last report in issue no. 8 listed three isolates from pools of *Culex tritaeniorhynchus* complex collected in the first half of August in villages near Lahore, West Pakistan. One additional isolate, I-326, also from a pool of 100 female *C. tritaeniorhynchus* complex collected in the same area and in the same period has been obtained and identified. All four isolates have been identified as West Nile virus by hemagglutination inhibition and complement fixation tests.

A small series of mosquito pools collected and processed during 1963 failed to yield any isolates. During 1964, over 100,000 mosquitoes were collected and pooled for virus isolation in the Lahore area. Processing of this material is progressing slowly. Mosquitoes collected in 1964 were taken at Shahzada Village approximately 20 miles to the south of Lahore, the site of three previous West Nile isolations in 1962, at the banks of the Ravi River on the edge of Lahore and from the Changa Manga forest 56 miles southwest of Lahore. The forest at Changa Manga is the only one in the Punjab and is a cultivated forest planted 100 years ago. It covers about 20 square miles and abounds with birds and wildlife. The mosquito collections at Shahzada Village and at the Ravi bank yielded large numbers of *Culex*, principally members of the *C. tritaeniorhynchus* complex, while catches at Changa Manga were principally aedines.

Cattle biting, human biting, and cattle-baited trap collections of mosquitoes were conducted routinely at Changa Manga through 1964. Additionally, a sentinel animal program, employing chicks and suckling mice, was initiated there late in the year. Collection of sera and plasma from a wide variety of bird, rodent, bat, and other small mammal species was made throughout the year.

Five virus isolates have been obtained from pools of a soft tick, *Argas abdussalami* n. sp. collected in nests of the white backed vulture, *Gyps bengalensis*, and on the limbs of

trees frequented by this species. These isolates have not been characterized or identified. One virus isolate obtained from a specimen of acute phase serum taken from the wife of one of our staff in Lahore, following the onset of a febrile disease, has been identified by hemagglutination inhibition test as the Sicilian strain of sandfly fever.

During the summer of 1964 an extensive outbreak of a febrile disease resembling dengue broke out in the city of Dacca, East Pakistan. Hemorrhagic manifestations were uncommon if they occurred at all. This outbreak was investigated by a team from the Pakistan Medical Research Center, consisting of Drs. Philip Russell, Michael Gregg, John Gorham, Mohammed Aziz, and Mr. Richard Fisher. Attempts to isolate viral agents in suckling mice from a collection of 3,000 mosquitoes collected in Dacca during the epidemic were negative. However, Dr. Russell reports the isolation of 10 agents from acute phase sera of patients, employing tissue culture systems. These agents have not yet been identified.

A colony of _Culex tritaeniorhynchus_ was established at the Lahore center during 1964 and material was recently carried to Baltimore in an attempt to establish a subcolony for experimental studies at the School of Medicine. This subcolony is progressing nicely.

REPORT FROM DRS. LIM KOK ANN AND Y.C. CHAN
DEPARTMENT OF BACTERIOLOGY, UNIVERSITY OF SINGAPORE
SINGAPORE 3

Typing of Dengue Viruses by Precipitin Agar Gel Diffusion (Dr. Chan).

The microprecipitin agar gel diffusion technique has been found to offer a rapid method of typing the four dengue virus types 1 to 4. The test employed crude 20% suckling mouse brain virus suspension and mouse hyperimmune serum on microscopic slide. Homotypic precipitin reaction was consistently obtained when each dengue virus type was tested against all the four types of dengue antisera. Virus isolates at the third mouse brain passage level have been successfully typed by this test but negative precipitin reaction has also been obtained in some instances. The results have been submitted for publication.

Isolation of Japanese Encephalitis Virus from Human Blood (Dr. Chan).

In the course of routine virus isolation from the acute phase blood of patients with hemorrhagic fever or suspected hemorrhagic fever, a strain of Japanese encephalitis virus was isolated from a child who presented with a pyrexia of unknown origin in August 1964. The virus (S-705/64) was successfully re-isolated from the original serum stored at -20°C. The patient's convalescent phase serum showed specific complement-fixing, hemagglutination inhibition, and neutralization antibodies to his own virus and to Japanese encephalitis virus (Nakayama). No complement-fixing or hemagglutination inhibition antibodies to the four types of dengue viruses were demonstrated in both the acute and convalescent phase sera. The virus was identified as a strain of Japanese encephalitis virus by cross complement fixation and neutralization tests and by the precipitin agar gel diffusion test. This was the first Japanese encephalitis virus isolation from human blood in Singapore. A report of the isolation has been submitted for publication.

REPORT FROM DR. DORA TAN, INSTITUTE FOR MEDICAL RESEARCH KUALA LUMPUR, FEDERATION OF MALAYSIA

Ecological Study of TP21 (Langat) Virus.

In this vector-virus study, *R. sabanus* rats were selected as the laboratory host from which Langat virus might be transmitted in titres adequate to infect *Ixodes granulatus* larvae feeding on them.

Four seronegative *R. sabanus* rats were inoculated subcutaneously with 0.1 ml of 1,000 LD_{50} of infected mouse brain suspension (7th passage) of Langat virus. Two rats were bled through the outer canthus of the eye every other day for 20 days, alternating between the pairs. Approximately 0.5 ml of blood was obtained and diluted to 50% with normal saline. Two litters of suckling mice were inoculated intracerebrally with 0.1 ml of this blood

and observed for 21 days. Infected mouse brains were harvested and tested for presence of Langat virus by the complement fixation test. Results of this experiment indicated presence of circulating virus in the rats from the 2nd to the 10th day after inoculation.

After the viremic phase has been determined, approximately 150 *Ixodes granulatus* larvae from a female tick, previously determined to be free of Langat virus infection, were atttached to 2 *R. sabanus* rats 5 days following subcutaneous inoculation with 0.1 ml of 1000 LD_{50} Langat virus. A portion of the fed larvae was ground-up and inoculated into 2 litters of suckling mice and another portion was attached to 3 suckling mice. The mice were observed for 21 days after which the surviving mice were challenged with 100 LD_{50} Langat virus. An isolate confirmed by the complement fixation test to be Langat virus was obtained from 5 suckling mice inoculated with ground-up larvae which had fed on one of the inoculated rats. The other mice were found uninfected and no antibodies were detected by challenge experiments.

The tick experiment will be repeated on obtaining more *Ixodes granulatus* larvae.

REPORT FROM NAVAL MEDICAL RESEARCH UNIT NO. 2
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Report from Arthropod Tissue Culture Laboratory - Dr. Earl C. Suitor, Jr.

Work on improving the procedures for culture of the moth and mosquito cell lines is progressing. For example, Grace's line of *Aedes aegypti* cells has been adapted to a medium containing Grace's medium plus 10% fetal bovine serum with only 0.5% *Philosamia cynthia pryeri* hemolymph. These cells have a generation time of approximately 58 hours. Also in progress are studies on the physiology of these cells. Included are intracellular pH determinations by microinjection procedures, and studies of effects of insect hormones on cell multiplication. Culture of arbovirus in these cells has thus far proven difficult. Tests are underway to determine the optimal conditions for arbovirus growth.

During the course of arbovirus assay with other types of cells (avian and mammalian), it has been noted that both WEE and Sagiyama viruses will produce much larger plaques under an overlay containing agarose plus dextran, than under overlays containing either agar alone, agarose alone, or dextran plus agar. Although the number of plaques is not increased, the average plaque size may be doubled. Details of this work will be outlined in future exchanges.

Report from Virology Department - Dr. Howard M. Jenkin

Investigations first begun in 1962 to test for the presence of arbovirus antibodies in human populations in and around Djakarta, Indonesia, are continuing. Neutralization tests using cell cultures of a hamster embryonic diploid strain (NAMRU-2) and HeLa MA cells have shown that antibody against Chikungunya-like, Jap B-West Nile-like, and Bunyamwera-like viruses were present in the 497 sera examined. Dengue cell culture neutralization studies are in progress using plaque reduction and interferon plaque reduction tests in MK2 Cells (R. Hull, Eli Lilly Company) and hamster embryonic diploid (HEM).

Recent interferon Chikungunya plaque reduction studies of unpaired sera collected from an outbreak of Philippine hemorrhagic fever in Manila in August, 1964, showed that dengue 4 virus was the probable etiologic agent of the disease. Cross neutralization against many other Group B viruses did not occur. Parallel neutralization tests in mice did not distinguish between dengue 1, 2, 3, and 4 viruses.

A plaque assay system using the methyl cellulose technique of Schlesinger has been successful in plaquing dengue 2 and 4 viruses in HEM cells. Titer levels are $10^{6.5}$ and $10^{7.5}$ PFU/ml for dengue 2 and 4, respectively. Mouse titration levels of the same inoculum were 10^{7} and $10^{7.7}$ $MICLD_{50}$/ml indicating similar levels of sensitivity in both assay systems. Plaque sizes ranged from 0.5-1.0 mm in diameter. Dengue 1 and 3 have not produced plaques in this cell system as yet using similar starting mouse $MICLD_{50}$ inoculum levels as dengue 2 and 4 viruses.

Report from Entomology Department - Eugene M. Bravi, Lt, MSC, USN

An outbreak of Japanese encephalitis virus disease (JEV) occurred during July and August 1964 in Hsin Chu County in Northwestern Taiwan. A total of 76 hospitalized cases was reported in children between three and eight years of age. The first case appeared during a rapid rise in the mosquito population representing species of the *Culex vishnui* group and 16 days after primary virus isolation from extracts of pooled mosquitoes from this group. The mosquitoes were captured in the general vicinity of the residences of some of the cases. The result of the identification of the etiological agent is summarized in Figure I.

During the mosquito infection season, serial serum samples from pigs in the Hsin Chu area were taken and are being tested for JEV neutralizing antibody. Further investigation will involve evaluating the relative vector importance of the species within the *Culex vishnui* group.

Identification of Virus Isolates from Hsin Chu, Taiwan, 1964

Mosquito Pool No.[1]	Date Collected	Virus Titer[2] Log_{10}	Neutralization Index[3] Log_{10}
H-390	6-20-64	7.4	3.4
H-462	7-4-64	8.2	2.7
H-463	7-4-64	8.5	4.0
H-464	7-4-64	7.7	3.6
H-478	7-6-64	6.5	2.8
H-487	7-6-64	8.5	3.1
H-491	7-8-64	8.0	3.1

1. Isolated in infant mice by injection of 0.02 ml mosquito pool extract intracerebrally.

2. Determination in adult Smith strain mice by injection of 0.03 ml of 10% suckling mouse brain suspensions intercerebrally.

3. JEV-NAK mouse immune serum vs. isolate.

REPORT FROM DR. LEON ROSEN, PACIFIC RESEARCH SECTION NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, HONOLULU, HAWAII

Recent activities of the Pacific Research Section, NIAID, have included investigations on an outbreak of dengue which began in French Polynesia in September 1964 (or possibly earlier). Studies in this area have shown that the last epidemic of this disease had occurred 20 years previously and that there was no serologic evidence of human arbovirus infection since that time. The diagnosis in the present epidemic was confirmed by rises in HI titer against the four dengue serotypes with the highest serum titers noted against types 3 and 4. To date, serum specimens for virus isolation have been collected from 25 patients.

The present disease in French Polynesia is relatively mild and no fatal cases are known to have occurred. Minor hemorrhagic manifestations were seen. Persons who had dengue in the 1944 outbreak have also acquired the disease in the current epidemic. As of December 1964, the disease was limited to areas where *Aedes aegypti* is abundant.

Preliminary testing of sera collected in New Caledonia and the New Hebrides in 1963 by the HI technique has revealed evidence of recent human group A infections in both areas. No evidence of group B infection after 1944 was found in either area.

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

The demonstration of Japanese B encephalitis virus antigen in human brain by fluorescent antibody technique (S. Hotta, A. Ohyama, and Y. Yasui).

In summer of 1964, a fairly large epidemic of JBE broke out in the Osaka-Kobe-Himeji district of Japan Mainlands. Nine cases diagnosed clinically as JBE were subjected to necropsic observations, and the fluorescent antibody method was applied using brain tissues*.

Anti-JBE hyper-immune serum** was obtained by injecting a male monkey with infected mouse brain homogenates of the Nakayama or JATH strain. Gamma globulin was separated,

which was then conjugated with fluorescein isothiocyanate. The brain tissues were frozen and cut at thickness of 6-10 microns in a cryostat at -20°C. The sections were fixed in cold acetone for 5 minutes. The direct method of staining was applied. The sections were covered with conjugated antiserum in an ice box at 4°C overnight and washed in buffered saline (pH 7.2).

In addition to the fluorescent antibody experiments, isolation of virus was attempted by injecting mice intracerebrally with the remaining parts of tissues.

Results obtained are as follows:

Patient No.	FA	Virus Isolation
1	+	+
2	+	+
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	+	+

Demonstration of JBE virus antigen in brain tissues by fluorescent antibody technique parallels well isolation of virus from the same materials.

*Kindly supplied by Drs. S. Yamagami, S. Sugiyama, and Y. Hironari of Momoyama Hospital, Osaka; Drs. T. Yamamoto and C. Tsunoda, of Municipal Central Hospital, Kobe; as well as Dr. E. Mizuno of Mitachi Hospital, Himeji.

**Kindly supplied by Drs. Nobuo Kusano and T. Aoyama, Institute of Infectious Diseases, University of Tokyo.

Further experiments on physico-chemical properties of dengue virus hemagglutinin (S. Hotta, M. Takehara, and T. Matumura).

Studies on partial purification and fractionation of type 1 dengue virus hemagglutinin were further extended (refer to: S. Hotta and T. Matumura, Arbovirus Infoexchange No. 10, pp. 12-13, October 1964).

Hemagglutinin of dengue virus Mochizuki strain was separated apparently into 3 components: slow-sedimenting (Hs), rapid-sedimenting (Hr), and more rapid-sedimenting (Hd)*.

Buoyant density values of 3 components obtained by CsCl equilibrium density gradient centrifugation at 35,000 rpm for 40 hours at 4°C were 1.19 (Hs), 1.24 (Hr), and 1.29 (Hd), respectively.

Most of mouse infectivity was found in Hr and Hd, and only less than 1% in Hs. HAU/mouse LD_{50} ratios of each component obtained in limited experiments were 43.4 (Hs), 1.2 (Hr), and 0.4 (Hd).

Heat stability of each component was different; it was found that Hs and Hd were comparatively heat stable, whereas Hr was more heat sensitive than the former two. Each component was completely inactivated by digestion with trypsin or pancreatic lipase at 37°C for 30 minutes.

Hs was apparently undetectable in "crude" samples subjected to the same centrifugation procedures; Hs was found in samples treated with ethanol or protamine. Two possible explanations for this finding were: 1) Hs might be "released" from virus particles by purification treatments; or 2) ethanol or protamine might remove "inhibitor(s)" against Hs. Further experiments are being planned to determine which is more likely.

No such components as described above were found in materials derived from control non-infected mouse brains.

Cultivation of dengue virus in chick embryo skin-muscle and African green monkey kidney cell cultures (S. Hotta, H. Aoki, and Y. Shimazu).

Mouse-adapted dengue virus (type 1 Mochizuki strain) showed a prolonged maintenance or a weak multiplication in primary

*"HA-dense", designated arbitrarily by the reporters.

trypsinized chick embryo skin-muscle (CESM) cell cultures. When the dengue virus of the same strain was successfully passed through primary trypsinized Japanese monkey (Macacus fuscatus) kidney (MK) cell cultures for more than 20 continuous passages, this proved to multiply significantly better in CESM cultures, compared with inoculum consisting of mouse brain homogenate virus. Viral yields were apparently better when the cultures were incubated at 25°C, compared with incubation at 30°C. No cytopathic effect was observed in infected CESM cultures.

Both MK culture-passaged and mouse brain-adapted virus strains multiplied well in established cell line cultures from kidneys of the African green monkey (Cercopithecus aethiops) showing significant sytopathic effect. It was observed that the cellular degeneration was never total but destruction and restoration of cellular sheets occurred intermittently and active virus was continually detected in the culture fluid for more than several months. "Virus carrier state" or "persistent infection" in this system of cell cultures was evident. Similar results were obtained with mouse-passaged yellow fever virus 17D strain.

REPORT FROM DRS. GEORGE C.Y. LEE, GEORGE E. KENNEY, AND J. THOMAS GRAYSTON

DEPARTMENT OF PREVENTIVE MEDICINE, UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE, SEATTLE, WASHINGTON

We have investigated the utility of continuous Syrian hamster cell strains for production and assay of high titered Japanese B encephalitis virus (JEV). Two cell strains "HL" from 1-day-old hamster lung and "HSS" from 12-day embryonic hamster skin were derived and maintained in serial passage. Cytopathic effect (CPE) developed rapidly in these cell strains when they were infected with JEV. Virus titers obtained in hamster cells strains ranged from 10^{8}-$10^{9.5}$ when measured by either plaque assay or suckling mouse infectivity. A variety of other cell lines and strains were tested in parallel, these included primary hamster skin, liver, lung, and kidney as well as primary human embryonic kidney, primary porcine kidney, primary chick embryo, and primary duck embryo. Varying lesser grades of CPE were obtained in the other cell systems tested with virus titers ranging from 10^{6} - $10^{8.5}$. Human diploid cell strains and primary hamster skin or kidney produced virus titers equivalent to continuous hamster cell strains but hemagglutinin yield was lower. Comparative

maximum hemagglutinin titers were 1:48 from primary hamster skin, 1:768 from continuous hamster embryonic skin (HSS), and 1:12 for human diploid strains.

Plaque assay: Large (3-5 mm) well defined plaques were produced in 4-5 days in both HL and HSS cell strains. Comparative assay of continuous hamster cell strain propagated virus indicated that both plaque assay and suckling mouse infectivity assay measured virus infectivity equivalently. This relationship did not change with serial passage of virus in continuous hamster cell strains, indicating that attenuation or "adaptation" was not occurring in this system.

Conditions for plaque assay:

a) Nutritional: Glutamine was required for plaque production in a complex medium of lactalbumin hydrolysate, yeast extract, and calf serum solidified with 0.6% agar (Difco "Noble").

b) pH: Optimal initial pH of overlay was 7.1-7.4.

c) Adsorption: Plating efficiency was maximal when adsorption was performed at 40°C. Half of the total potential plaque-forming units had already attached by 30 minutes. Below 40°C plating efficiency was directly related to temperature. Indirect adsorption experiments indicated that 50% of total virus in the inoculum was attached by 1 hour at 36 C while 35% was attached by 1 hour at 30 C.

d) Diluent for adsorption: Plating efficiency was enhanced by the presence of proteins such as bovine albumin, gelatin, or calf serum in the attachment medium. Likewise, slower removal of virus (indirect adsorption) from inoculum was demonstrated in non-protein containing medium indicating that attachment of virus was the factor, not merely protection of virus from inactivation.

e) Thermal inactivation: The half life of JEV as measured by plaque assay of virus incubated in non-wettable plastic tubes was: 30 C : 160 minutes, 36 C : 140 minutes, and 40 C : 100 minutes.

f) Linearity of assay: The relationship between number of plaques and relative virus dosage was linear.

Preliminary trials of vaccines in mice employing killed virus obtained in the described systems indicated that vaccine potency was more directly related to hemagglutinin titer than to infectivity.

REPORT FROM CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH,
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DIRECTOR, ROCKEFELLER FOUNDATION ARBOVIRUS STUDY UNIT,
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There were 10 confirmed cases of WEE encephalitis in man in California in 1964. The first case occurred in Chico, Butte County, with onset July 27th. This was the only case in July. There were 4 cases in August and 5 in September. There were 3 cases in Yolo County and one case each in Tehama, Butte, Lake, Yuba, Sutter, Sacramento, and Plumas Counties. The latest onset date was September 24th and that was the case in Plumas County. There were 31 confirmed cases of WEE encephalitis in horses and 5 presumptive positive cases, one of which was a fatal case which occurred in Sonoma County in October and where a single blood specimen showed a very high titer for WEE antibody by the CF test. Among the cases showing a 4-fold rise of CF antibodies in comparison of acute and convalescent serum specimens, the first case occurred in mid-July; the acute serum specimen was taken on July 12th. There were 5 cases in July, 16 in August, 5 in September, and one in October, where the approximate date of onset was given. There were single cases in Shasta, Sutter, Colusa, Placer, Merced, and Fresno Counties, 2 in Glenn, 9 in Butte, 2 in Yolo, 6 in Sacramento, 4 in San Joaquin, and 3 in Stanislaus Counties. The location of the other cases has not been determined. According to this information, one can say that the WEE virus was active in the northern half of the Central Valley of California, with an unusually late seasonal pattern. The only concentration of cases was that in Chico, Butte County. WEE virus was isolated from tree squirrels found sick in this county on September 23, 1953, and July 21, 1955, and from ground squirrels found sick on July 29, 1955, and September 13, 1962. Most of the cases of horse encephalitis occurred in ponies kept in small paddocks at ranch type homes in and about Chico. All were associated with irrigated pasture and the presence of large trees, used as roosting trees by migratory blackbirds. The single

human case of WEE encephalitis occurred in the city of Chico at a home adjacent to a walnut grove and there was a small cattle feed lot nearby which attracted migratory blackbirds. Flocks of several thousand blackbirds were observed feeding in pasture fields which were being flooded. Sparrow and house finch populations were low. There are numerous walnut orchards in and around Chico and the area is known also for the very large and ancient oak trees which are found along the water courses. No tree squirrels were seen during a 2-day visit to the area, indicating a very low tree squirrel population. A flock of several hundred crows was observed roosting in the trees of a park in the city of Chico. A few blackbirds had been found dead under oak trees in the park in Chico prior to the investigation of the outbreak. There had been no obvious increase in the mosquito population. The association of cases of encephalitis with large trees used as roosting sites for flocks of migratory Brewer's blackbirds indicates that this wildlife host could have served as both the source and the means for amplification of the WEE virus in the community.

There was only one confirmed case of St. Louis encephalitis in man in California in 1964. This case was staying at Blythe, Riverside County, during the period immediately prior to the illness which began August 21, 1964. There were 2 cases of encephalitis in horses where a presumptive diagnosis of SL encephalitis could be made by the serological tests of acute and convalescent serum specimens. One of these was from Imperial County. This horse showed a drop in CF antibodies for SL virus in a comparison of the acute and convalescent serum specimens taken in August. This case of horse encephalitis occurred in the Imperial Valley of Southern California near to the case of SL encephalitis in man. The other case of possible SL encephalitis in a horse occurred in Sacramento County. There was only a 2-fold rise of antibody for SL virus by the CF test. All the acute blood specimens from cases of horse encephalitis were tested for virus by the infant mouse inoculation test and they were all negative for virus.

There were 8 cases of Colorado tick fever, 4 with onset in June, 3 in July, and one in August. These cases were exposed by tick bite in the mountains. CTF virus was isolated from the blood clot of a _Peromyscus maniculatus_

wood mouse collected May 7, 1964, in Modoc County. CTF virus was isolated from a pool of *Dermacentor andersoni* ticks collected in Modoc County June 17, 1964, and from a pool of *Dermacentor andersoni* and another of *Dermacentor occidentalis* ticks collected in the Clear Lake area of Klamath County, Oregon, just across the border from California, on June 17, 1964. The tick collections made in 1964 were a continuation of the collections made over several years in an effort to delineate the zone of activity of CTF virus. The isolation of CTF virus from a wood mouse adds another species to the list of small mammals found infected with this virus in nature in California. The virus has been isolated also from wood rats, golden-mantled ground squirrels, chipmunks, and pocket mice. Of 21 mother mice that were held for challenge with CTF virus after the litters of infant mice had been inoculated and died of CTF virus from natural sources, 5 resisted intracerebral challenge with the Florio adult mouse adapted CTF virus. This shows that they had become infected with CTF virus from contact with the infected infant mice. It was not possible to determine whether those which were resistant had eaten infected infant mice. The dead mice were ordinarily removed for specificity tests when the mice were checked.

Four pools of *Dermacentor* ticks held for more than 4 years in glass sealed tubes in a dry ice chest were found positive for CTF virus in 1964. These were from known foci of activity of the virus. Additional pools of ticks from the same areas are being held for testing after longer periods of storage.

REPORT FROM THE ARBOVIRUS UNIT
DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF ARIZONA
TUCSON, ARIZONA

Laboratory studies during the 1964 hot season in Southern Arizona have yielded no virus isolations from arthropods, mammals, or birds, indicating probably a low level of arbovirus activity in this area. Four flocks of sentinel chickens have been maintained in the Santa Cruz Valley in the vicinity of Tucson. To date, 389 birds, 11,012 mosquitoes*, and 50 pools of ticks have been collected for laboratory tests. A few sera from horses suspected by attending veterinarians of having viral encephalitis

*Of these, 8,455 were *C. quinquefasciatus* and 2,494 were *C. tarsalis*.

were tested. Small numbers of human sera for suspected cases of CNS infections were likewise checked for arbovirus antibodies; all horse and human sera were negative to St. Louis and WE. Results of serological studies on the sentinel flocks will be reported in the next Infoexchange.

Snake Cell Culture Studies.

Early in November of this year, we began preliminary studies on the propagation of cell cultures from snake tissues. This work was prompted partially by the studies of Gebhardt et al. (1) in which western encephalitis (WE) virus was isolated from a number of wild snakes and also by some of the previous work by other investigators. Our initial aims are as follows:

1) To determine whether or not we could grow snake cells in culture, and, if so, under what conditions would they grow.

2) To determine if such cell cultures could be propagated serially.

3) To determine the susceptibility of primary and/or establish snake cell cultures to WE and other arthropod-borne viruses.

Our results to date are summarized below.

Preparation of Cell Cultures.

The snake used was a female garter snake (Thamnophis marcianus) which weighed 127.2 gm. and measured 582 mm. in total length. It had been caught in the Tucson area. The snake was anesthetized by means of Nembutal and a blood sample was taken by cardiac puncture. An aliquot of the blood has been tested for viral activity and was negative after two passages in primary hamster kidney tissue culture, and also a single passage in suckling mice.

A second portion of snake blood was allowed to clot and the serum thus obtained was processed for and tested by the HI method against WE antigen and A169 antigen (A169

is a local isolate for an SLE-like virus). No HI antibody was demonstrated to either antigen. The tissues taken for cell culture studies were kidney, lung, and ovary. These tissues were minced, washed with Hank's BSS, and then digested at 4°C. with 0.25% trypsin. Three hours after trypsinization began, turbidity was noted with all three tissues so these trypsin suspensions were decanted and centrifuged for five minutes at 500 rpm and used for cell culture preparations as indicated below.

Fresh trypsin was added to the remaining tissue and trypsinization, with agitation, was continued overnight at 4°C. Centrifugation and cell culture preparations were performed with these suspensions in the same manner as was performed with the three-hour trypsinization material.

Cell packs were re-suspended in medium consisting of Eagles MEM with 15% fetal calf serum added. Penicillin and streptomycin in concentrations of 100 units/ml. were included in the medium. Tube cultures (0.5 ml.) and 2 oz. prescription bottles (5 ml.) cultures were prepared, cultures were incubated at 24°C. and at 37°C. and were observed daily for growth. Both three-hour trypsinization and overnight trypsinization cultures were prepared from lung and kidney while only three-hour trypsinization cultures were obtained from ovary tissue. No turbidity was noted after the overnight trypsinization of the ovary tissue so this material was discarded.

Cell Growth.

The following chart summarizes the results of cell culture studies as observed to date.

Tissue Used	Trypsinization Time @ 4°C.	Incubation Temperature	Observations
Lung	3 hours	24°C	Slow growing, fibroblast-like cells, cells first noted at 7 days, confluent growth 19 days, good cell sheets still present at 28 days. Medium not changed, pH 7.2-7.4. No passage attempts.
Lung	24 hours	24°	Similar to above, growth first noted at 5 days.
Lung	3 hours	37°C.	No growth.
Lung	24 hours	37°C.	Scattered fibroblast-like cells observed 7 days, no growth remaining by 10 days.
Kidney	3 hours	24°C.	Islands of epithelial-like cells first observed at 5 days, good growth by 13 days, have held for 27 days. No passage attempt.
Kidney	24 hours	24°C.	Similar to above. Second passage successful, solid sheets of epithelial-like cells obtained.
Kidney	3 hours	37°C.	Slight growth noted on 5th day, lost by 10th day.
Kidney	24 hours	37°C.	Epithelial-like growth noted on 5th day, heavy growth by 7th day, lost on 10th day.
Ovary	3 hours	24°C.	Epithelial-like growth observed at 4 days, heavy growth by 7 days. Secondary passage successful, although solid cell sheets not obtained.
Ovary	3 hours	37°C.	Slight growth observed at 5 days, attempted passage of heaviest cultures not successful.

The results described above are from a limited number of cultures as we did encounter some difficulty with contamination. It is evident, however, from these initial studies, that incubation at a lower temperature (24°C.) has given more promising results than at 37°C. and also that the cultures do not require media changes of pH alterations at frequent intervals. We plan to continue studies with other garter snakes with other snake species testing other combinations of growth conditions.

Viral Studies:

Preliminary studies with limited numbers of primary cultures have indicated that WE virus causes CPE in snake kidney cells (3+ reading at 48 hours using a 10^{-2} dilution of virus) and a lesser response in ovary cells (1+ under similar conditions) The specificity of those reactions has not as yet been determined. Further studies are being planned and results will be reported at a later date.

Reference: Gebhardt, Louis P., G. John Stanton, Douglas W. Hill, and Glen C. Collett, 1964. Natural Overwintering Hosts of the Virus of Western Equine Encephalitis. New England J Med. 271:172-177.

REPORT FROM DR. VERNON SCOTT
THE UNIVERSITY OF OKLAHOMA MEDICAL CENTER
OKLAHOMA CITY, OKLAHOMA

Approximately 16,460 mosquitoes were collected from Cheyenne Bottoms, Kansas, in New Jersey light traps and identified to species. From 86 pools of *Culex tarsalis*, 8 pools of *Culex pipiens* and 10 pools of *Culiseta inornata* (approximately 50 mosquitoes per pool), 14 agents have been isolated which kill suckling mice following passage through a bacterial filter. Thirteen agents have been isolated which kill suckling mice but have not been filtered. One of these 27 agents has been identified as western equine encephalitis virus. More than 650 bird blood specimens have been collected for studying to

detect viral agents and specific viral (WEE, EEE, and SLE) antibodies. Twenty agents have been isolated which kill suckling mice on original passage. Further identification of these agents is underway.

Three sentinel flocks of chickens were placed at three different sites. These were bled periodically and studies with the hemagglutination inhibition test are underway at present to detect the presence of antibodies to WEE, SLE, and EEE viruses.

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

The summer and fall of 1964 in Texas was characterized by the simultaneous occurrence of an SLE outbreak in Houston and a predominantly WE outbreak centering in and around Hale County in the Texas High Plains. By far the largest number of cases occurred in Houston. Scattered SLE and WE cases occurred in several counties. Some of these SLE cases evidently acquired infection in Houston.

This department collaborated with the CDC in Atlanta in an investigation of the SLE outbreak in Houston and with the Greeley and Kansas City Field Stations in the Hale County investigations.

The HI test was utilized to good advantage for rapid early reporting and maintenance of interest of physicians in the investigations.

Serologically confirmed or presumptive WE infections of equines were found in many counties, in nearly every section of the state.

Both SLE and WE viruses were repeatedly found in _Culex_ mosquitoes. Other viruses were found in pools of mosquitoes. In summary, 19 virus isolations were obtained from 156 pools of mosquitoes from 8 counties. Some of the viruses remain to be identified.

REPORT FROM THE DISEASE ECOLOGY SECTION, USPHS, CDC, GREELEY, COLORADO; AND THE TEXAS STATE DEPARTMENT OF HEALTH, AUSTIN, TEXAS

I. Encephalitis Outbreak in Hale County, Texas.

During late July and early August, 1964, it became apparent that an outbreak of acute, febrile, central nervous system illness was beginning in the Texas Panhandle, with a number of cases being reported from Hale County where cooperative studies on encephalitis have been carried out during recent years. The Plainview-Hale County Health Department, the Texas State Department of Health, and the Greeley and Kansas City Field Stations of the Communicable Disease Center, USPHS, collaborated in an investigation of the outbreak in Hale County. The Kansas City Field Station was concerned primarily with investigating the etiology of cases which showed no WE or SLE antibodies, and which might be caused by enteroviruses. The results of their studies are not included in the present report.

A. Human Cases.

In and around Hale County, as in 1963, suspect cases began to build up in late July and early August, reached a peak in middle to late August, and declined in September. Reported suspect cases who resided within Hale County for at least one incubation period prior to onset totaled 70, and 8 suspect cases from surrounding counties came into Hale County for treatment. These cases, by week of onset, are shown in Figure 1. Etiology of these cases based on serological tests is shown in Table 1. Of the confirmed or presumptive cases, about 80 per cent were positive for WE and 20 per cent for SLE. Almost half of the cases for which adequate serum specimens were available were negative for both WE and SLE. These cases are currently under study by the Kansas City and Greeley Field Stations to determine if they were due to arboviruses other than WE or SLE, or to some non-arbovirus etiologic agent. Reports to the Texas State Department of Health show that the outbreak was not limited to Hale County, since numerous cases of WE and SLE were reported from other counties in the Panhandle area.

In addition to the serological tests, WE virus was isolated from the brain and spinal fluid of one fatal case, and from the brain of another fatal case. Age-specific attack rates for Hale County for confirmed and/or presumptive cases of

Fig. 1 SUSPECT ENCEPHALITIS CASES IN HUMANS

HALE COUNTY, TEXAS AND SURROUNDING AREAS

1964

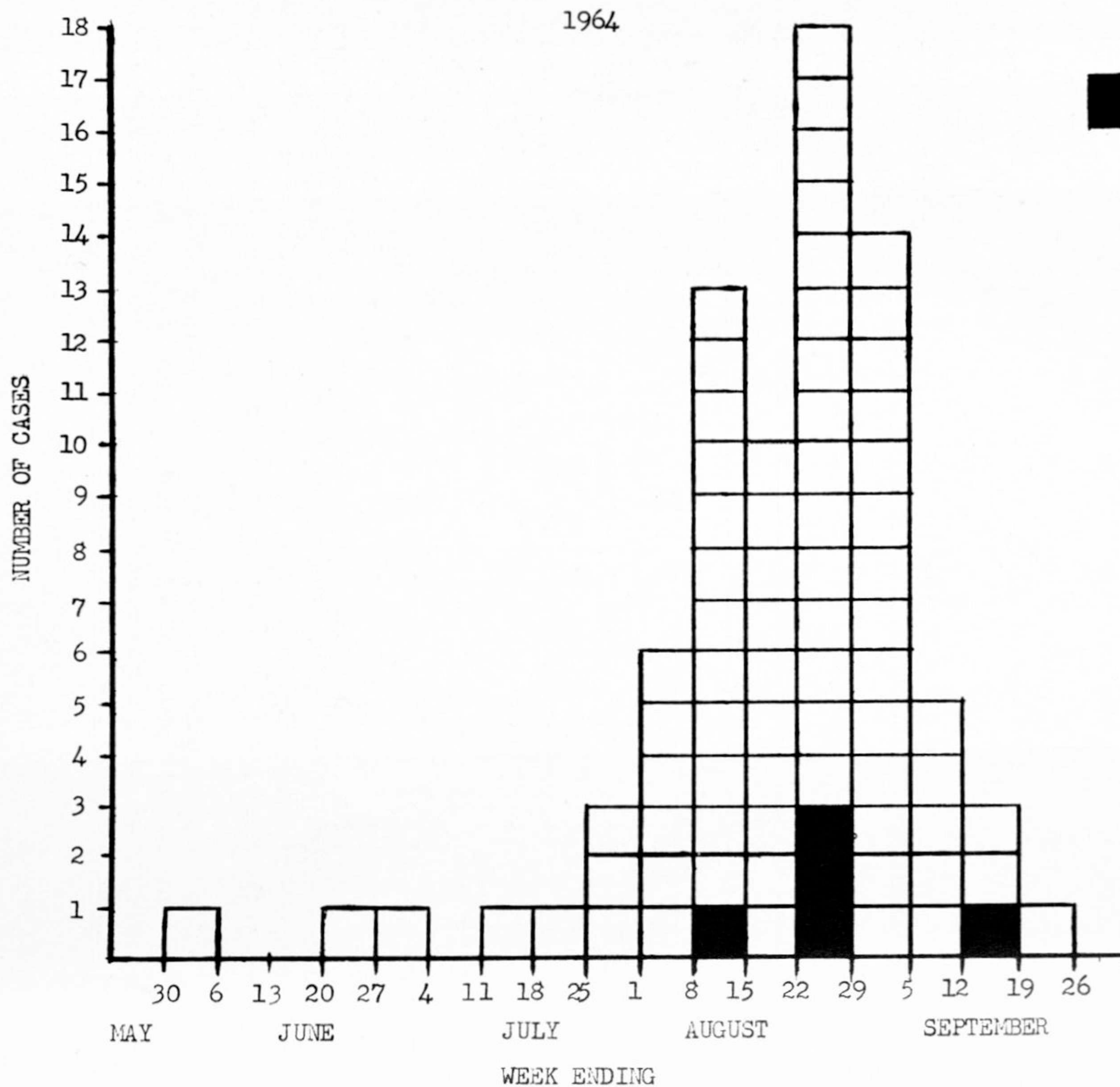


Table 1. Etiology of Reported Suspect Human Cases of Encephalitis Hale County, Texas, and Surrounding Areas, 1964
(Based primarily on HAI tests run in the Greeley laboratory)

Conf. or Presump. WE	Conf. or Presump. SLE	Equivocal WE	Equivocal SLE	Neither WE nor SLE	Specimens Incomplete or Inadequate for Classification
24	6	1	3	31	11

Note: One reported case removed from consideration, diagnosis equivocal--not acute, febrile, CNS disease.

One reported suspect case with presumptive serological evidence of <u>both</u> WE and SLE, not tabulated.

WE, and for those cases neither WE nor SLE, are seen in Table 2. As is usual with WE outbreaks, the attack rates were much higher in the 0-4 age group than in any of the older age groups. Of the 11 WE cases in the 0-4 age category, 6 were less than 6 months of age. Had population data for the 0-6 months age group been available, the attack rates for these infants would presumably have been far higher than for any of those shown. The overall attack rate for the 5 confirmed or presumptive cases of SLE which occurred in Hale County was 13.6 per 100,000.

Attack rates for Latin Americans were over 3 times as high as for Anglo Americans. Eight of the suspect cases were among Mexican nationals ("braceros") present in the country on special agricultural labor contracts. An accurate estimate of the number of braceros in the county during the outbreak is not available, but it is believed to range between 200 and 1,000. Even on the basis of the higher estimate, the attack rate for this itinerant group would be over twice that for the resident Latin Americans.

B. Serologic Survey of Horses.

Very few horse cases were reported in the Hale County area during the 1964 outbreak. Since WE virus outbreaks usually involve horses, a serologic survey was conducted from Hale County horses at the close of the outbreak. These animals had not had signs of encephalitis, nor were they selected because of any illness. Twenty of 23 yearlings had HI antibody to WE, and 14 of 16 two-year-old horses had WE antibody. Five of the 23 yearlings had HI antibody to SLE, and 9 of the 16 two-year-olds had SLE. In retrospect, it is apparent that many of the horses had become infected with WE or a related Group A virus during the 1964 outbreak; SLE virus or a related Group B virus was also active in the equine population, though to a lesser extent than WE.

C. Mosquito Population Index and Mosquito Infection Rates.

New Jersey light traps were used to determine the population indices of *Culex tarsalis* throughout the summer. The 1964 index was similar to that of several previous years (Fig. 2). A buildup occurred during June, reaching peak populations during July and August. By mid-August, a marked population decrease began, but large numbers of *C. tarsalis* were still active by mid-September. In spite of the continued availability of these mosquitoes, the WE infection rate for *C. tarsalis* dropped precipitously (Table 3) during late August and early September. The same phenomenon has been

Table 2. Age Specific Attack Rates, Encephalitis Outbreak, Hale County, Texas, 1964

Age Specific Attack Rates for Western Encephalitis

Age	Cases	Population*	Attack Rates per 100,000
0-4	11	4,704	234
**5-9	2	4,495	44
10-19	1	7,010	14
20-29	1	4,798	21
30-39	2	4,546	44
40-59	3	7,455	40
60+	-	3,790	-
	19	36,798	51.6

Age Specific Attack Rates for Reported Suspect Encephalitis Found Serologically not WE nor SLE

Age	Cases	Population*	Attack Rates per 100,000
0-4	8	4,704	170
5-9	5	4,495	111
10-19	6	7,010	86
20-29	5	4,798	104
30-39	4	4,546	88
40-59	1	7,455	13
60+	-	3,790	-
	29	36,798	79

*1960 census
**One case with serological evidence of both WE and SLE

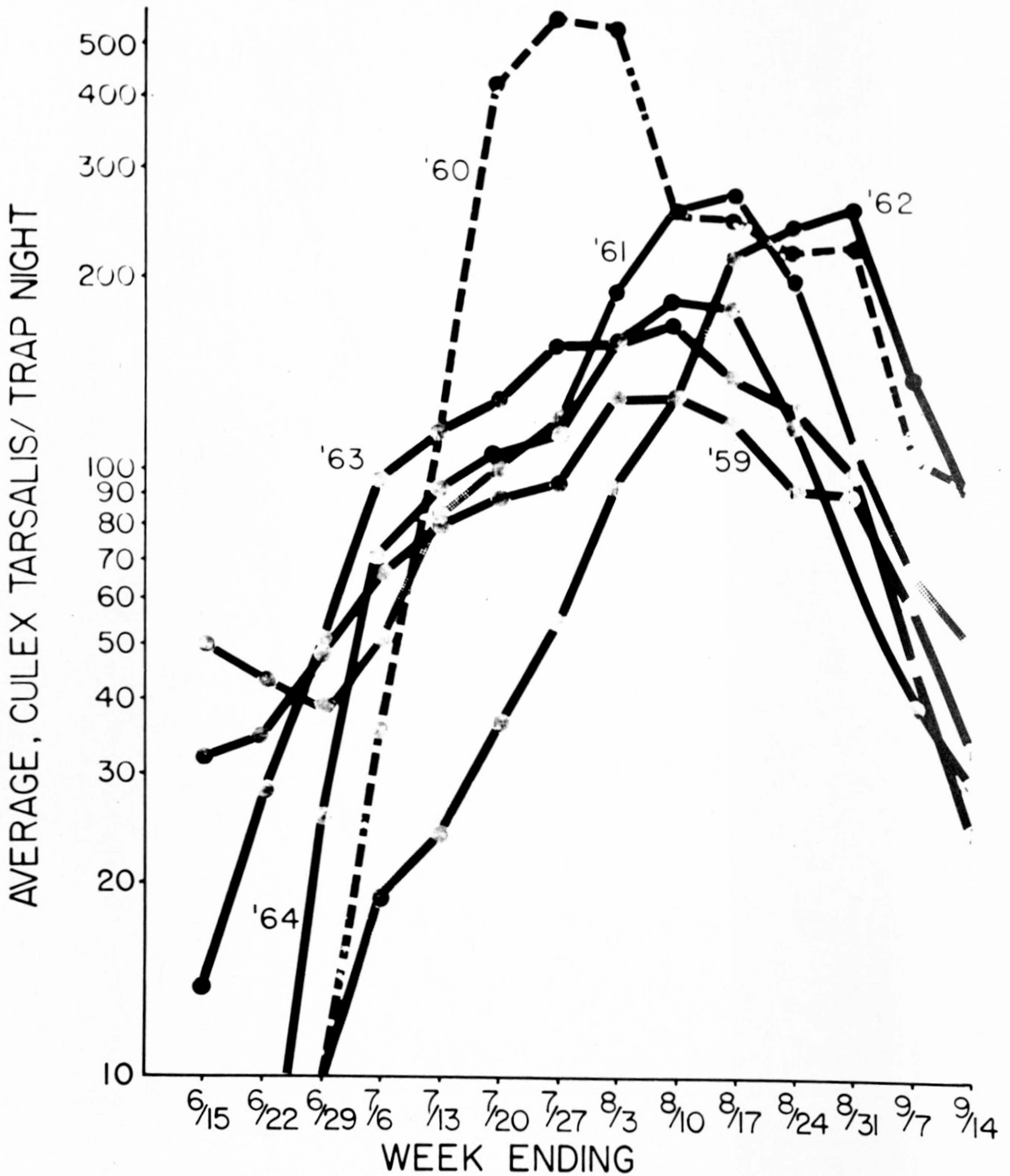


Fig. 2. Adult Culex tarsalis populations as measured by light traps, Hale County, Texas, 1959-1964.

Table 3. WE Isolations from Hale County, Texas, *C. tarsalis*, 1964

Collection Dates	Number Mosquitoes	Number Pools	WE Isolations / 1000 Mosquitoes
27 Jul - 31 Jul	750	15	14.66
3 Aug - 6 Aug	520	12	9.62
11 Aug - 14 Aug	704	15	12.78
17 Aug - 21 Aug	1876	41	12.79
24 Aug - 26 Aug	1232	33	9.74
31 Aug - 4 Sep	858	31	8.16
7 Sep - 10 Sep	449	23	6.68
14 Sep - 18 Sep	384	27	2.6
21 Sep - 24 Sep	118	7	0

observed in previous years in the same Texas area. This suggests that the source of WE virus for the mosquito population may be curtailed at some point during the epizootic, which would limit the period of virus dissemination in nature.

Other viruses were isolated from *C. tarsalis* including Hart Park, Turlock, and as yet unidentified virus agents. One isolation of WE was made by the Texas State Health Department from *Aedes nigromaculis*. Most of the mosquito species other than *C. tarsalis* are yet to be tested.

D. Feeding Patterns of Culex tarsalis.

Engorged *Culex tarsalis* mosquitoes were collected from the outbreak area in Hale County from the latter part of May until the latter part of September. The specimens were sent to Dr. Connie Tempelis at the University of California School of Public Health in Berkeley, and he ran micro-precipitin tests to determine the source of the mosquitoes' blood meals. The results (Figure 3) show that the mosquitoes fed predominantly on chickens and wild birds during the early part of the season, but that there was a marked increase in feeding upon mammals during the peak of the transmission season. Similar patterns have been observed in Colorado and Kern County, California. This feeding pattern would permit a maximum amplification of virus during the spring period when mosquitoes were feeding on nestlings and other non-immune birds, and the subsequent rise in mammal feeding during late July and early August would correspond with the major period of transmission to humans and equines. The increased mammal feeding together with rising immunity in the bird population would also help explain the decline in mosquito infection rates at the end of the season.

E. Sentinel Chicken Flock Serology.

Two sentinel flocks of chickens were maintained throughout the summer in Hale County. By the end of the outbreak period, 96 percent of the chickens in each flock had HI antibody to WE virus and 46 and 56 percent of the chickens in the 2 flocks had HI antibody to SLE These are the highest transmission rates which have been observed in the area during the 1958 to 1964 period that sentinel flocks have been maintained. The relative activity of WE and SLE in the sentinel flocks also corresponded fairly closely with the human cases as will be seen from the following tabulation:

Human Cases	
Percent Confirmed or Pres.	
WE	SLE
80	20

Sentinels	
Percent HAI Positive	
WE	SLE
96	51

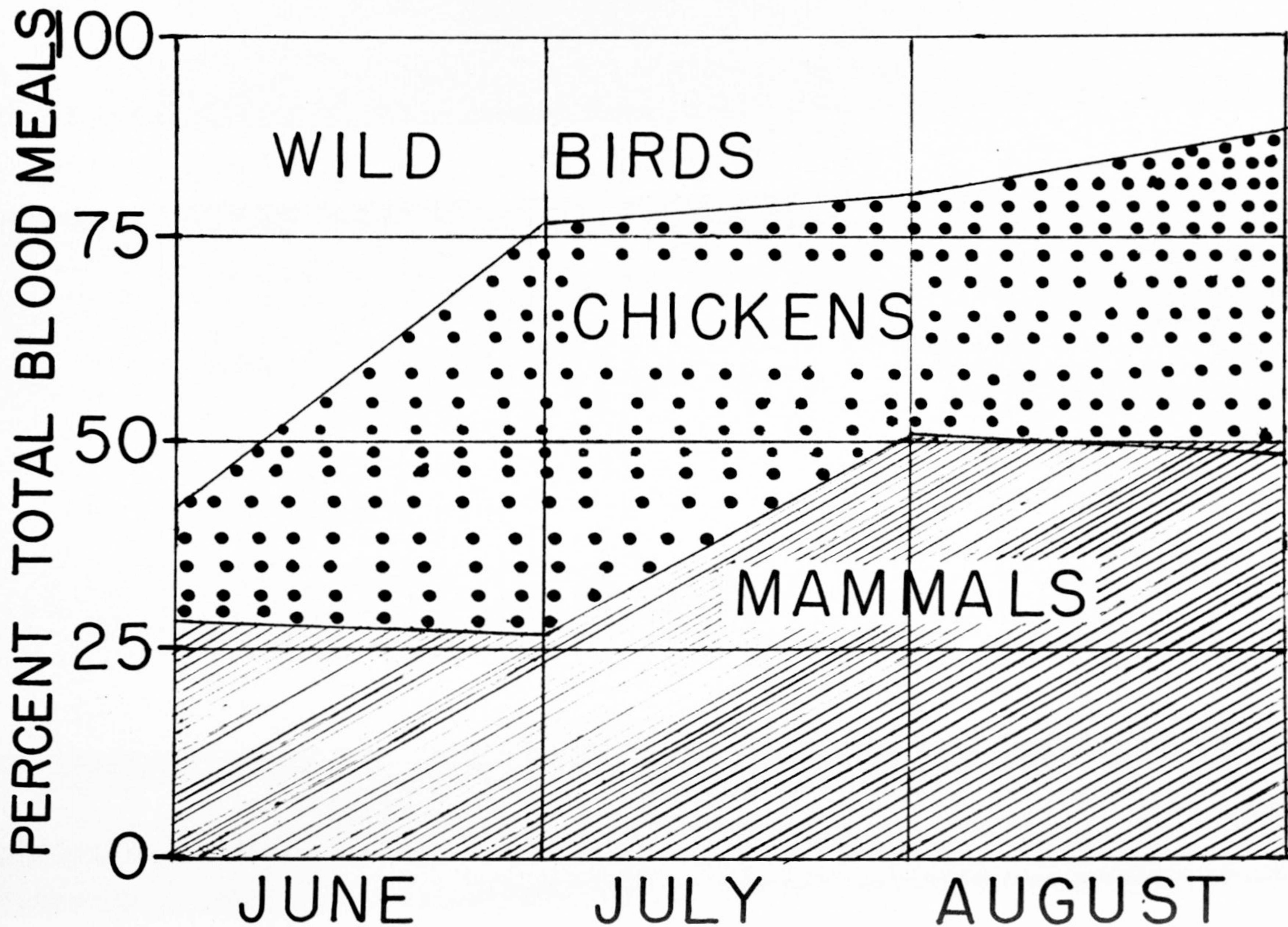


FIG. 3. SOURCE OF BLOOD MEALS OF CULEX TARSALIS COLLECTED FROM HALE COUNTY, TEXAS, 1964

REPORT FROM THE DISEASE ECOLOGY SECTION, USPHS, CDC GREELEY COLORADO; AND THE COLORADO STATE DEPARTMENT OF HEALTH, DENVER, COLORADO

Encephalitis Outbreak in Eastern Colorado.

An outbreak of acute febrile central nervous system disease began on the eastern slope of Colorado during the first week of August, 1964. Human cases as well as horse cases were reported during the outbreak. The Colorado State Department of Health and the Disease Ecology Section, CDC, collaborated in the ensuing investigation.

A. Human Cases.

A Total of 104 suspected human cases were reported, exclusive of patients who subsequently developed mumps or polio antibody rises and other diseases. There were 3 deaths associated with the outbreak. Cases occurred in 22 of 63 counties, on the western as well as the eastern slope of the state. About half of the cases were reported from 4 eastern slope counties (Table 1). It will be noted that the attack rates were progressively higher in the counties with smaller populations. This is in keeping with the usual rural and suburban pattern of encephalitis outbreaks where *Culex tarsalis* is the primary vector. Serologically confirmed cases of both WE and SLE occurred from the first week of August to the fourth week of September (Table 2). The age distribution of the WE cases (Table 3) agreed with the generally accepted idea that clinical cases of this disease are most often seen in young children. The SLE infections were well distributed among the age groups. Of the 98 cases from whom serum specimens were obtained, 13 were considered either confirmed or presumptively positive with the HAI for WE antibody, and 25 gave similar evidence of SLE infection. CF tests are not yet complete, but it appears that at least 50 per cent of the cases will be negative for WE and SLE. This is the usual picture in arboencephalitis outbreaks in which a large proportion of suspect cases must be listed as of unknown etiology. This offers a challenge to public health workers concerned with encephalitis outbreaks. Studies are underway to test acute and convalescent sera against a battery of known arboviruses.

Table 1. Attack Rates of Encephalitis in Four Colorado Counties, 1964 Outbreak

County	Population (1960 Census)	Etiology of Cases			Attack Rates per 100,000
		WE	SLE	Unidentified	
Denver	493,887	2	3	10	4
Adams	120,296	3	4	3	8
Larimer	53,343	0	6	2	15
Otero	24,128	3	4	4	45

Table 2. Date of Onset of 69 Suspect Encephalitis Cases, Colorado, 1964

Week of Onset	Serologic Diagnosis		
	WE*	SLE*	Unknown
July 14-20			1
Aug 1-6	1	1	1
7-13	3	2	2
14-20	3	3	0
21-27	2	2	4
28-31	0	2	3
Sept 1-6	1	2	6
7-13	0	3	0
14-20	1	4	3
21-27	2	3	3
28-30	0	0	0
Oct 1-6			9
7-13			1
21-27			1
Totals	13	22	34

*Confirmed and presumptive HAI positives

Table 3. Age Distribution of 72 Suspect Cases of Encephalitis, Colorado, 1964

Age	Serologic Diagnosis		
	WE*	SLE*	Unknown
0-4	4	2	5
5-9	1	2	3
10-14	1	0	2
15-19	0	3	8
20-24	2	1	3
25-29	0	1	2
30-34	0	1	3
35-39	1	2	3
40-44	0	1	3
45-49	3	3	1
50-59	0	3	1
60-69	0	3	3
70-79	0	1	0
Totals	12	23	37

*Confirmed and presumptive HAI positives

B. Horse Cases.

Many horse cases were reported to the Colorado State Department of Health, although most of these were not laboratory confirmed. Among those horse cases from which sera were obtained, the Fort Collins sera provided an interesting study group (Table 4). Of 14 suspect horses, 9 showed diagnostic HAI titer rises and 3 were considered presumptively on the basis of a single specimen with HAI titer of 1:160 or higher and clinical signs consistent with encephalitis. Four horses had low titers of antibody which inhibited SLE antigen. The apparent predominance of WE activity in the equine population was in contrast with the etiology of the human cases which appeared to have been caused primarily by SLE virus.

C. Mosquito Data.

The population index for *C. tarsalis* suggested that this mosquito was no more abundant in Weld County than it has been in previous years (Figure 1). The population had already reached peak levels by early July, and declined rapidly during late August and early September. Thus, the seasonal population curve did not differ markedly from that of most previous years.

In spite of this similarity in *C. tarsalis* populations, 1964 was a year of significantly more WE and SLE transmission. Comparatively few mosquito pools were tested, but the number of WE isolations from Weld County *C. tarsalis* clearly indicates the extent to which this virus was active in this area (Table 5). The failure to isolate SLE virus from the rural areas was probably related to the relatively few mosquito pools collected during the outbreak. The *Culex pipiens-quinquefasciatus* complex, so important in urban SLE epidemics, was scarce in the Fort Collins area at the time of the outbreak.

The source of the *C. tarsalis* blood meals was determined throughout the summer by Dr. Connie Tempelis at the University of California School of Public Health, using a microprecipitin test. As in the Texas outbreak area, *C. tarsalis* fed predominantly on birds during June and early July. At the peak of the transmission season during late

July and early August, there was a sharp increase in the per cent of mosquitoes feeding upon mammals. As discussed under the Texas outbreak, the feeding pattern may have important epidemiological significance.

D. Sentinel Chickens.

Two sentinel chicken flocks were maintained in Weld County. The Timnath flock, located between Greeley and Fort Collins, had HI antibody rates of 50 per cent for WE and 35 per cent for SLE. The Greeley flock had HI rates of 14 per cent for WE and 34 per cent for SLE. These higher rates for SLE activity are in general agreement with the relative numbers of WE and SLE cases in humans.

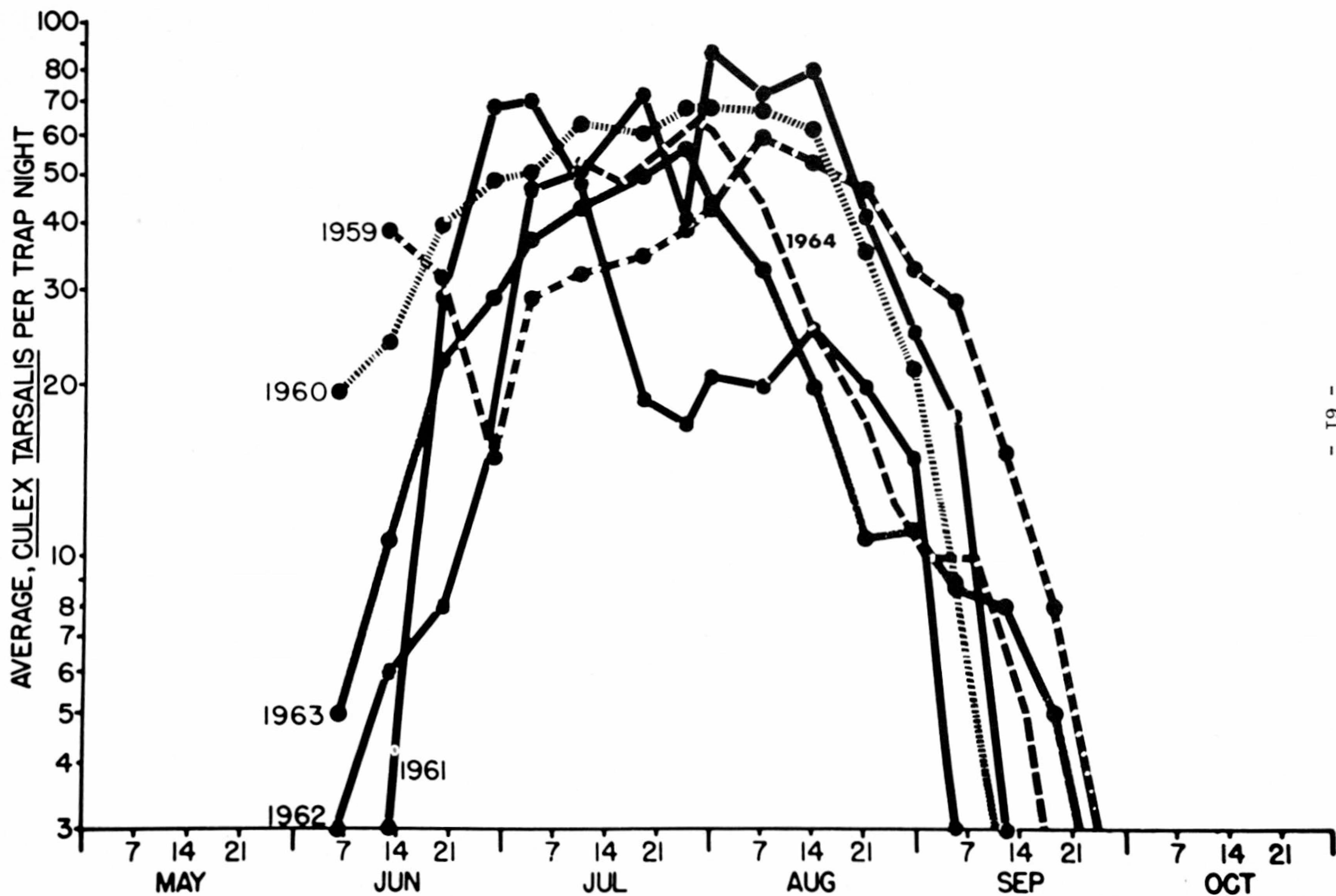
Table 4 Results of HAI tests of sera from suspect cases of equine encephalitis, Colorado, 1964

Area	Number tested	Number positive		
		WE		
		Confirmed	Presumptive	SLE
Fort Collins	14	9	3	4
Montrose	1	1	0	
Greeley	2	0	2	
Carbondale	1	0	0	
Totals	18	10	5	4

Table 5. WE Isolations from *C. tarsalis*, Colorado, 1964

Collection Dates	Number Pools	Isolations		
		WE	Turlock	Agent (Unidentified)
3-7 Aug	12	2	1	0
11-14 Aug	3	1	0	0
19 Aug	1	1	0	0
23-25 Aug	3	2	0	0
2 Sept	2	1	0	1
Totals	21	7	1	1

Fig. 1 Adult Culex tarsalis populations as measured by light traps, Greeley area, Colorado, 1959-1964



REPORT FROM DR. CARL M. EKLUND
ROCKY MOUNTAIN LABORATORY, HAMILTON, MONTANA

TICK TRANSMITTED VIRUSES

Colorado Tick Fever (CTF)

I. HUMAN DISEASE

Virus was isolated from the blood of 40 patients. As shown in Table 1, exposure was in western states where there are known endemic areas.

Patients

Colorado	Idaho	Montana	Nevada	Oregon	So. Dakota	Wyoming	Total
2	9	8	6	4	3	8	40

Date of Onset

April	May	June	July	August	Total
2	16	12	9	1	40

Onset was primarily in May and June. As far as is known, the disease seen was mainly of the diphasic febrile type. One person was said to have a stiff neck. No special attempt is made to locate cases of CTF so the number of isolations obtained must represent only a very small proportion of the total incidence of CTF.

II. MAINTENANCE OF CTF AND POWASSAN VIRUSES.

Studies were again carried out in Spearfish Canyon, Black Hills of South Dakota, with the main purpose of determining the longest possible time that a nymph-small mammal-larvae virus cycle or nymph-small mammal-nymph cycle can operate in the maintenance of these two viruses.

During the period April 27 to May 10, 176 D. andersoni nymphs and only 2 larvae were collected from small mammals. Conditions at this time were obviously unfavorable for a nymph-mammal-larva virus cycle but favorable for a nymph-mammal-nymph cycle. The situation was different for Ixodes spinipalpis; 13 nymphs and 38 larvae were collected, offering a reasonable opportunity for a nymph-mammal-larvae virus cycle as well as a nymph-mammal-nymph cycle to operate. A similar situation prevailed for the Ixodes ochotonae-angustus complex. Studies during previous summers had shown that there were optimum opportunities for exchange of viruses between nymphs and larvae during July and August for both Dermacentor and Ixodes spp.

Arthropod	April-May	August-Sept.	Oct.-Nov.	Hosts	Virus Isolations
Dermacentor andersoni	215 adults 176 nymphs 2 larvae	8 nymphs 60 larvae		Flagged Clethrionomys, Microtus, Marmota, Eutamias, Glaucomys, Peromyscus, unidentified	13 CTF 2 Powassan
Ixodes spinipalpis	1 adult 15 nymphs 38 larvae	46 nymphs 43 larvae	1 adult 1 nymph	Clethrionomys, Microtus, Peromyscus, Eutamias, Glaucomys	
Ix. ochotonae-angustus	9 adults 13 nymphs 19 larvae	3 adults 20 nymphs 33 larvae	1 nymph	Clethrionomys, Microtus, Peromyscus, unidentified	
Ix. soricis	1 larva			Sorex	

Collections during late August and early September still showed relatively favorable conditions for a nymph-mammal-larvae virus cycle in the case of D. andersoni and favorable conditions in the case of Ixodes spp. During late October, conditions were unfavorable for any virus

virus cycle because of small numbers of both ticks and mammals. On the basis of virus isolations from small mammals, number of small mammals, and the relative proportion of larvae to nymphs observed during the past three summers, July and August are the months with optimum conditions for disseminating virus in the nymph-mammal-larvae virus cycle, but the nymph-mammal-nymph cycle can take place from the first of May to the first part of October.

Rodent Blood Specimens

Rodent	April-May	August-Sept.	Oct.-Nov.	Virus Isolations
Microtus	31	19	12	
Eutamias	10	31	2	
Peromyscus	9	71	100	
Clethrionomys	7	21	1	2 CTF 1 Powassan 1 unidentified
Mustela		3		
Marmota	1			
Neotoma	1			
Procyon	1			
Sorex	1			
Zapus	1			

Although 6 isolations of Powassan virus have now been made from adult D. andersoni no evidence of human infection has yet been found in the Black Hills area in spite of the fact that CTF infection is relatively frequent there.

A technique for the collection of all active stages of unengorged D. andersoni has been developed during this past tick season by Dr. Richard Garcia. The basic principle employed in the technique was the use of CO_2 (dry ice) as an attractant. A piece of dry ice weighing approximately

two pounds was placed on a wire mesh platform for a period ranging from 1 to 2 hours. Two systems were employed to recover the ticks from the immediate vicinity of the gas source. The first system consisted of a white flannel flag, 3 x 3 ft., which was laid over the substrate surface after removal of the dry ice. The cloth was gently pressed down and then turned over. The ticks adhering to the cloth are removed, and the procedure repeated until subsequent efforts yielded no ticks. The second system was to spread a 3 x 3 ft. white flannel cloth on the ground in the desired area. The dry ice and platform were then placed in the center of the cloth. After the period of gas release was completed, the cloth was picked up and the ticks were removed from the surfaces of the cloth. Adults and nymphs were easily removed by hand or with forceps while the larvae required the use of an aspirator.

Preliminary results indicate that the technique was particularly more sensitive than conventional flagging for adult ticks during the early and late portions of the tick season. During March, early April, July, August, and September, adult ticks were collected with CO_2 when only an occasional individual was detected by previously flagging the areas.

The system is capable of collecting large numbers of ticks at an individual site. One hundred seventy-eight adults and 26 nymphs were collected during a 2-hour period at one CO_2 site in an area of high tick abundance in north-central Montana.

Nymphs were first observed around CO_2 sites during early May; however, routine sampling was not started until mid-July in Lost Horse Canyon. Nymphs collected at 250 sites from mid-July through the first week in August averaged approximately 1.4 nymphs per CO_2 site. The number collected per site was highly variable, ranging from 0 to 15. The higher frequencies were most often observed in areas particularly suited for small mammal activity, such as spaces among rocks and at the base of cliffs. After the first week in August, nymphal activity was greatly reduced and they were not collected after mid-September. Nymphs collected at 285 sites during this period averaged only .15 per CO_2 site.

A few larvae were first observed on cloth August 6th; however, this probably did not represent their initial period of activity.

Approximately 650 larvae were collected from Lost Horse Canyon from mid-August to mid-September using a total of 185 CO_2 sites. Larvae were not collected after October 5th.

Virus isolation from unengorged D. andersoni, Lost Horse Canyon, Bitterroot Valley *

Stage	Total No.	No. pools	No. isolations	Estimated Inf. Rate
Adult	200	20	13	9.7%
* Nymph	350	35	2	.6%
Larvae	650	26	0	-

***There are 2 other isolations of virus from 2 pools of 12 and 13 respectively, collected in Chaffin Creek Canyon, Bitterroot Valley.**

The infection rate in adults was consistent with rates obtained by previous investigators in this canyon.

GROWTH OF CTF VIRUS IN D. ANDERSONI

When larvae were permitted to engorge on infected hamsters, virus was first detected on the 3rd day of feeding and reached a maximum titer of $10^{-4.95}$ and maximum amount of virus of $10^{3.96}$ LD_{50} per pool of 10 larvae on the 8th day after start of feeding. Soon after molting to nymphs (which began on the 20th day after start of engorgement) a titer greater than $10^{-5.64}$ and an amount of virus greater than $10^{4.46}$ LD_{50} were reached. Virus was first detected in the salivary gland on the 23rd day of the experiment. When infected nymphs were allowed to engorge on normal hamsters 78 days after start of the experiment, there was a further increase in amount of virus which reached a peak of greater than $10^{5.87}$ LD_{50} on the 96th day. On the 110th day, at which time adults were present, there had been a drop in average amount to $10^{4.4}$ LD_{50}. The main purpose of the experiment was to show that when D. andersoni are infected as larvae, they will molt to nymphs with a large enough amount of virus to be efficient transmitters of

virus to small mammals. When infection first occurs during the nymphal stage, the peak amounts of virus, $10^{5.5}$ or > LD_{50} are noted just prior to and after molting to adult stage. Larval ticks took up roughly 30 times their weight in blood. The average amount of blood taken by a larva is .58 mg. The nymphs took up over 50 times their weight of blood with an average of 10 mg per nymph.

MITES IN RELATION TO WEE, SLE, AND CTF VIRUSES

The vector potential of a large mite, Haemogamasus liponyssoides liponyssoides was studied by Dr. Garcia in relation to WEE, SLE, and CTF viruses. This mite is an obligate parasite which obtains its blood meal by piercing the skin of a variety of rodent and insectivore hosts. Three stages in the life cycle, the male, female, and duetonymph, require a blood meal. This mite was selected for study because it is an obligate bloodsucking parasite of small mammals and it and closely related forms have a wide geographic distribution.

Cultures were reared on suckling mice until each contained several hundred mites. Suckling mice (3-4 days old) were inoculated with .05 cc of a 10^{-3} brain suspension of the virus and used as the infective source for the mites. Mice inoculated with WEE were held 24 hours while those inoculated with SLE and CTF were held 48-96 hours before exposure to the mite cultures. Cultures of mites were exposed continuously to infected suckling mice for periods ranging from 1 to 6 weeks at room temperatures (70 $\pm$ 5°F). Several cultures of mites were maintained for each virus.

All three viruses were detected routinely in mites if they were ground and inoculated into suckling mice within a few hours after engorgement. The amount of virus decreased rapidly and was never detected after approximately 24 hours at room temperature.

Transmission was observed occasionally when infected mice and normal mice were added to the mite cultures simultaneously. It was not clear whether this was a form of mechanical transmission by the mites due to interrupted feedings, or direct transmission from mouse to mouse by cross contamination of the wounds produced by the mites.

Under the experimental conditions thus presented, the evidence indicates that this mite is not important in the maintenance of these arboviruses.

MOSQUITO TRANSMITTED VIRUSES

No western or St. Louis virus activity was detected. Virus was not isolated from 3164 *Culex tarsalis* collected at Vale, Oregon, or 974 *C. tarsalis* collected at Bismarck, North Dakota. No antibodies were found in 23 sentinel chickens at Vale, Oregon; no virus could be found in the blood of 16 garter snakes collected at Vale, Oregon, although bleedings were done before and after refrigeration.

The summer was cold and C. tarsalis did not appear to be very abundant.

REPORT FROM RESEARCH STATION, ENTOMOLOGY SECTION, CANADA DEPARTMENT OF AGRICULTURE, LETHBRIDGE, ALBERTA; AND PROVINCIAL LABORATORY OF PUBLIC HEALTH, EDMONTON.

From the beginning of June until the middle of October, 1964, mosquito collections were taken at various locations in the irrigated areas and in some locations outside the irrigated areas of the southern region of Alberta. Mosquitoes were collected also in the northern region of the province. The mosquitoes were identified, quickly frozen, and brought to the Provincial Laboratory, where they were kept at -70°C until tested.

So far, 144 pools of mosquitoes out of 270 received have been tested for the presence of arboviruses (see Table 1). Each pool consisted of an average of 30 mosquitoes, the number varying from 20 to 50. Eight infant mice, 1 to 2 days old, were inoculated intracerebrally and kept for observation during 14 days. If any mice were thought to be sick, a portion of the original specimen was reinoculated into a further litter of infant mice. Brain passages of the sick mice were performed only if the mice in the second litter also became ill.

No isolations of virus have been recorded to date. However, a number of pools are "suspicious" and are still under investigation. The failure so far of attempts to isolate arboviruses from Albertan mosquitoes may be correlated with the comparative absence this year of arbovirus disease in animals and man.

(Drs. J.A. Shemanchuk, R.H. Robertson, and O. Morgante)

TABLE I

LOCATION AND SPECIES OF MOSQUITO POOLS TESTED

	A. campestris	*A. cataphylla*	*A. dorsalis*	*A. excrucians*	*A. fitchii*	*A. flavescens*	*A. punctor*	*A. spencerii*	*A. vexans*	*A. inornata*
Southern region of Alberta	1	3	22		1	11		9	35	13
northern region of Alberta		20		7	11		10		1	

REPORT FROM DRS. J. MCLINTOCK, A.N. BURTON, J.G. REMPEL, AND H. DILLENBERG,
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AND THE PROVINCIAL LABORATORY, DEPARTMENT OF PUBLIC HEALTH, REGINA, SASKATCHEWAN.

Arbovirus Studies in Saskatchewan, 1963.

Study of the data collected during the 1963 epidemics has continued, particularly with reference to the sequence of events.

In Saskatchewan in 1963, mean weekly temperatures reached their summer maxima during the weeks ending August 5 and 12, and the populations of *Culiseta inornata* and *Culex tarsalis* followed, reaching their summer peaks about a week later, around the middle of August. The *Aedes dorsalis* populations behaved differently; at the six trap sites, this species reached its summer peaks earlier (weeks ending July 15 to August 5) and usually before the summer temperature peak had been reached.

Among the univoltine species, most reached their summer peaks, at any one trap site, within one or two weeks of each other, but between the six traps their peaks were spread over five weeks, from the week ending July 15 to August 19.

An account of the WEE virus isolations obtained in Saskatchewan in 1963 has been given in Issues 9 and 10. In Table 1, the virus isolations are listed in chronological order with the dates of the first reported horse and human cases. Here we see that the virus was present in nature at least as early as June 22, reached its activity peak in the mosquito and bird populations from about August 6 to 11, and we could no longer detect the virus in the mosquitoes and birds after August 21. Apparently the virus started to overflow into the horse and human populations well before the activity peak in nature was reached, before mean weekly temperature peaks were attained, and before *Culex* and *Culiseta* reached their population peaks.

The peak of the WEE horse epidemic occurred during late August and in man early in September. If we assume an incubation period of about 11 days in man and horses, the peak attack rates in horses probably occurred during the second and third weeks of August and in man in the last week of August and the horse and human infection peaks would therefore have followed both the mosquito population and virus activity peaks in nature.

With the exception of the June 22 isolation from C. tarsalis at Outlook, all the virus isolations from mosquitoes taken in our routine survey traps were obtained when the populations of the species were at, or near, their summer peaks. The last infected mosquitoes taken came from the Saskatoon trap, from August 15 to 21, but cases were reported late in September; hence, infected mosquitoes must have been on the wing at least into the first week of September. On September 30, C. tarsalis was still being taken in all traps except Melfort, and C. inornata in all traps except Craik.

The indicated sequence of events was as follows: Temperature Peak ---> Virus Activity Peak in Nature ---> Mosquito (Culex and Culiseta) Population Peak ---> Peak in Horse Epidemic ---> Peak in Human Epidemic. By the time the human epidemic was well underway, the populations of C. tarsalis and C. inornata had already started to decrease.

The isolation of WEE virus from C. tarsalis taken at Outlook on June 22 is of interest for several reasons. It was the earliest isolation of WEE virus that we had yet obtained in Saskatchewan. It was obtained from a pool containing only four females of the species; and since the first males of C. tarsalis did not appear in the Outlook trap until July 4, these females were probably overwintered specimens that had picked up the virus during or after their emergence from hibernation. Finally, the highest mean daily temperature preceding the date of capture was 73°F (23°C) (on June 20) and the next highest 70°F (21°C) (on June 17). In the 10 days preceding June 22 there were 18 day-degrees above 65°F (18°C).

1964:

Mosquitoes were much less numerous in Saskatchewan during the summer of 1964 than in 1963. For the summer as a whole, Aedes vexans was the most abundant species of mosquito.

Forty-one horse cases of WEE were reported and only one human case.

We again operated six routine mosquito light traps with five in the same locations that they occupied in 1963. We also maintained six sentinel chicken flocks that were bled twice a month and mosquitoes were collected on each occasion when a flock was visited for bleeding. Otherwise, our procedures were the same as those followed in 1963.

Weather

Apart from two short periods of severe temperatures, one in the third week of November (24°F (31°C) - 28°F (33°C) below zero) and the other from the 10th to the 21st of December (25°F (32°C) - 35°F (37°C) below zero), the winter (November to March) of 1963-1964 in Saskatchewan was generally mild and precipitation was normal to slightly above normal.

All of April and the first three weeks of May were warm, with below normal precipitation in April and above normal precipitation in the warm weeks of May. The weather then turned cold and dry and some parts of central Saskatchewan received no further significant amounts of rainfall until the third week of July.

From June 1 to September 30 (the period of mosquito trap operation), the general course of the mean weekly temperature curves was similar in all trap districts but tended to be higher in the south. There was a period of above normal temperatures from the weeks ending July 13 to August 10, but the other summer weeks had normal to below normal mean temperatures. During the last week of August, the weather turned cold and wet, a situation that lasted till the end of September and brought an early end to the mosquito season.

Precipitation was more variable between different locations. In the south, there were moderate rains during the weeks ending June 15 and 22 that were not shared by the remainder of the province. With the exception of Outlook, which had a dry summer, all districts had a period of wet weather later in the season, beginning in the month from July 13 to August 24, depending on location, and lasting for 3-8 weeks. During the hot weather of July and early August, much of the rainfall in all districts occurred in thunderstorms, and it was this combination that was responsible for the outbreak of A. vexans.

Mosquito Abundance.

As indicated by comparison of the catches in the five traps that ran in both years, the mosquito population of 1964 was only 1/6th that of 1963. A. vexans formed more than half (54%) of the total number of mosquitoes taken (8% - 63%)*, followed by C. inornata (8% - 51%) and A. dorsalis (2% - 20%). C. tarsalis was in sixth place (1% - 14%), followed by A. flavescens (1% - 11%). The last four species are those from which the virus of WEE has been isolated in Saskatchewan.

In Table 2, the dates of first appearance of males and females of C. tarsalis in the six traps are listed, the trap locations being arranged in order from north to south down the list. With two exceptions, the dates are earlier as we proceed down the list. In the first five traps, the females all appeared later this year than in 1963 (this was the first year for the Weyburn trap); the males were later at Saskatoon and Delisle and earlier at the other three locations. At Weyburn, the males appeared before the females and at Swift Current almost at the same time, which suggests that the overwintered populations of C. tarsalis might have been very small in these two districts. The month of June was cold, particularly during the last three weeks, when mean minimum weekly temperatures in our trap districts ranged from 3.0 to 11.3 degrees (F) lower than in 1963; this was probably the principal reason for the failure of C. tarsalis to build up a large population later in the season.

A. flavescens was almost twice as abundant as in 1963; it was present when the traps started, increased through June and July, but decreased rapidly in August in spite of the heavy rains in that month. C. inornata was the most abundant species in the traps in each month except July, when it was exceeded by A. vexans; and in August A. vexans was still the most abundant species in the Weyburn trap.

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*Range of percentages of the total number of mosquitoes taken in each of the six survey traps.

Virus Activity.

With our miscellaneous collections of mosquitoes (collections made by methods other than light trap) on a more routine basis, we were able to obtain many more specimens by these methods than in 1963. To date, our two isolations of WEE virus from mosquitoes in 1964 have been obtained from our miscellaneous collections, one from *A. flavescens*, taken at Wawota on June 23, and the other from *C. tarsalis*, taken at Canora on August 15, both while investigating reported cases in horses. The virus was also isolated from the blood of a nestling barn swallow taken on the same day and on the same farm where the infected *A. flavescens* were found.

In Table 3, the incidence of WEE antibodies that developed in indicator, or sentinel, chicken flocks in 1963 and 1964 are compared. In 1963 the infections were acquired between July 31 and August 21;in 1964, they were acquired between August 6 and 21.

From the data accumulated this far, it is apparent that, along with the reduced number of cases in horses and humans, there was much less WEE virus activity in mosquitoes and birds in Saskatchewan in 1964.

Table 1.

WEE Virus Isolations from Mosquitoes and Birds in Saskatchewan, 1963.

Date Collected	Location	Species	Number of Isolations
June 22	Outlook	*Culex tarsalis*	1
July 4	Fairmount	Swainson hawk	1
July 27	Outlook	*Culiseta inornata*	2
July 29	First horse case reported.		
July 31 - Aug. 3	Forestry Farm, Saskatoon.	*Culex tarsalis*	1
Aug. 1	Brock	House sparrow	1
Aug. 2	First human case reported.		
Aug. 3 - Aug. 6	Melfort	*Culex tarsalis*	1
Aug. 4 - Aug. 6	Craik	*Culex tarsalis*	1
Aug. 5 - Aug. 6	Forestry Farm, Saskatoon.	*Aedes dorsalis*	1
Aug. 6	Beadle	House sparrow	2
Aug. 6	Brock	*Culex tarsalis*	2
Aug. 6	Brock	*Culiseta inornata*	1
Aug. 6	Kindersley	*Culex tarsalis*	1
Aug. 6	Kindersley	Chicken	1
Aug. 8	Brock	House sparrow	1
Aug. 8	Outlook	*Culex tarsalis*	2
Aug. 8	Milestone	*Culex tarsalis*	1
Aug. 9	Brock	House sparrow	1
Aug. 9	Milestone	House sparrow	1
Aug. 8 - Aug. 12	Craik	*Culex tarsalis*	1
Aug. 10	Outlook	*Culiseta inornata*	1
Aug. 11	Outlook	*Culex tarsalis*	1
Aug. 13	Moose Jaw	House sparrow	5
Aug. 15 - Aug. 21	Forestry Farm, Saskatoon.	*Culex tarsalis*	1
		Total	31

Table 2.

Dates of First Appearance in Light Traps of *Culex tarsalis*

Saskatchewan, 1964.

Location	Females	Males
Melfort	July 16 (June 29)	July 20 (July 27)
Saskatoon	July 12 (July 10)	July 22 (July 14)
Delisle	July 12 (June 29)	July 16 (July 14)
Outlook	June 25 (June 22)	June 29 (July 4)
Swift Current	June 24 (June 18)	June 23 (July 10)
Weyburn	June 27	June 18

Dates in brackets are those for 1963 .

Table 3.

WEE Antibody Incidence in Indicator Chicken Flocks,

Saskatchewan, 1963, 1964

(White Leghorn and Grey Line Strain)

	1963			1964		
LOCATION	NUMBER POSITIVE	NUMBER NEGATIVE	% REACTORS	NUMBER POSITIVE	NUMBER NEGATIVE	% REACTORS
Aberdeen	8	16	33.3%	0	24	0%
Estevan	22	1 (1 died)	95.7%	7	17	29.16%
Kindersley	21	2 (1 died)	91.3%	5	19	20.8%
Dafoe (1963)	4	19 (1 died)	17.4%			
Outlook (1964)				1	23	4.16%
Saskatoon	3	14 (1 died)	17.6%	2	21 (1 died)	8.69%
St. Walburg	0	24	0%	2	22	8.33%

a) Each flock consisted of 24 birds with the exception of the 1963 Saskatoon flock which had 18.

b) All birds were WEE antibody negative when placed in the field.

REPORT FROM DR. ROBERT P. HANSON
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Over 30 thousand arthropods were collected in central and southern Wisconsin during the summer of 1964. The collections contained 24,757 mosquitoes, 4,908 Culicoides, 135 black flies, 123 tabanids, 144 stomoxys, 563 hippelates and 1,140 ticks.

Thirteen agents lethal for suckling mice were isolated from 1,032 pools of insects. Seven isolates came from _Aedes vexans_. (July 15-20 Aug.). Two isolates came from _Aedes trivittatus_ (July 7-31). One isolate came from a pool of _Aedes triseriatus_ (16 Aug.) one from a pool of _Culex restuans_ (25 June), one from a pool of _Culex_ species (15 Aug.) and one from a pool of _Hippelates_ species (6 July).

Identification of the isolates has not been completed. Antisera prepared to four of the isolates did not react with western, eastern, or St. Louis encephalitis antigens. One isolate reacts to high titer with trivittatus virus antigen.

Three Silverwater viruses have been isolated from the rabbit tick (_Haemaphysalis leporis-palustris_) material collected near Alberta, Canada during 1964. Two of these isolates were made from tick egg pools (July 5 and July 8) and one isolate was made from a pool of nymphal ticks (July 18). The fourth isolate, a member of the California encephalitis virus group was made from an _Aedes_ spp. pool collected on July 31.

Populations of biting diptera in Alberta were greatly reduced during the early summer. The mild winter and a low spring precipitation resulted in a very dry April, May and early June.

More than fourteen hundred cold blooded vertebrates were collected in Wisconsin during 1964. No agent lethal for chicken embryo has been isolated from blood or tissues of 427 specimens so far tested.

It would appear from the negative results in a series of transmission trials with western, eastern and California encephalitis viruses, that _Haemaphysalis leporis-palustris_ and _H. chordeilis_ ticks are probably not involved in the epidemiology

of these three arboviruses. A cone-shaped trap with a circumference of 12 feet that utilized a carbon dioxide bait was found to be as effective and often superior to conventional light traps and animal shelters in capturing a representative segment of the hematophagous arthropod population in a series of trials in Wisconsin.

Over 4,000 serum samples from Wisconsin, Georgia, Texas, Indiana, Alberta and Panama were tested for virus neutralizing antibodies for one to ten arboviruses. California encephalitis virus neutralizing substances were found in sera obtained in all areas and in a large percentage of the individuals of such species as deer (213/566), foxes (26/49), man (135/906) and snowshoe hares (88/227). Of possible significance was the detection of virus neutralizing substances for this virus in blood of migratory waterfowl (22/232). Venezuelan encephalitis virus neutralizing substances were found in a significant number of deer (47/377) and other animals from a coastal area in Texas. Antibody to vesicular stomatitis, New Jersey serotype, was detected in 26 percent of 520 negroes from Sapelo Island on the coast of Georgia. Ten percent of the negroes had antibodies to California encephalitis virus and two percent had antibodies to eastern encephalitis virus. St. Louis or St. Louis-like antibodies were found in 10 of 906 human sera, 13 of 566 deer sera, 11 of 227 snowshoe hare sera and 70 of 217 wild turkey sera.

Serological indices of cattle that were fully susceptible, partially resistant and fully refractory to challenge with vesicular stomatitis virus, New Jersey serotype were determined using (1) the mouse protection test, (2) the tissue culture metabolic inhibition test, and (3) the virus neutralization test. Virus neutralizing titers obtained in embryonating eggs and in mice did not differ significantly. High virus neutralizing titers (1,000 to 100,000 LD_{50} neutralized) did not necessarily indicate that the animal would be refractory to challenge. Animals with high mouse protection titers (greater than 2) always had high virus neutralizing titers and were refractory to challenge. The titers in the metabolic inhibition test usually paralleled the mouse protection titers.

In Alberta during 1964 antibodies to arboviruses either appeared in the snowshoe hare population late in the season or not at all. California virus antibodies were found in 80 percent of the population in August, a level that was reached in June in 1962 and 1963. There was no evidence at any time of western or eastern encephalitis virus activity.

In 1963 all hares were reactors to western encephalitis by June. Later in 1963 an epidemic of western encephalitis occurred in man and horses in Alberta south of the study area. There was no epidemic in 1964. Eastern encephalitis was last detected in the area in 1962, when both the virus and antibodies were demonstrated.

REPORT FROM DR. EDWIN W. JENNEY
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The screening of insect pools for the isolation of vesicular stomatitis (VS) virus has consisted of insect collections made in the summer of 1963 during a large VS outbreak of the New Jersey type, a small collection from Georgia in the summer of 1964, and of a collection made in Colorado during the summer of 1964. Indiana type VS which occurred in Colorado during 1964 had not been diagnosed in the United States for eight years.

A total of 90 pools of Georgia insects collected during the summer of 1963, mostly of the families Culcinae and Tabanidae, have been inoculated intracerebrally into 1-day-old suckling mice. New Jersey type vesicular stomatitis was isolated and reisolated from a pool of *Psorophora confinnis* and from a pool of *Chrysops* collected during active VS infection on a farm; however, both of these insect pools were ground on the same day and are the only pools of 90 from which VS virus was isolated. As both types of VS virus had been used in the laboratory for serological tests on the day these two pools were prepared, it appears that the diluent was contaminated.

Three other virus isolations made from the Georgia 1963 collections were all from *Culex salinarius*. Two of these pools were collected on the same farm on consecutive days. These three isolates have not been identified. New Jersey and Indiana types of vesicular stomatitis virus have been eliminated and alkaline aqueous antigens have not shown hemagglutination with goose red blood cells. One of three isolates kills 1-day-old but not 3-week-old suckling mice. Two of the three isolates have been reisolated from the original mosquito pool and the third had a similar pattern on both inoculations. (One new caretaker recorded, but did not salvage dead mice.)

The average survival time of two of these pools on isolation was 6-7 days with 8 for the third. By the fifth passage, the average survival time was 4 days for all 3 isolates.

Seventeen lots of Chrysops and other Diptera mostly Tabanidae collected in Georgia during the summer of 1964 have been negative. Collections were made on farms which have frequently had vesicular stomatitis but very little VS activity was reported from the Newman, Georgia area during 1964.

Forty pools have been made from 67 lots of insects received from Colorado during 1964. Thirty of these lots are negative. Eight have not been inoculated long enough for conclusions. Of two which require more passages, one may contain an agent of mouse origin.

The 40 Colorado pools consisted of 67 lots of insects which include the following: Tabanus sp., Chrysops sp., Simuliidae, Siphona irritans, Musca sp., Stomoxys calcitrans, Fannia sp., Reduviidae, Muscina sp., Culicinae and Ceratopogonidae.

Eight pools were made from light trap collections made in the Ames area by a graduate student in medical entomology at Iowa State University. An isolation has been made and reisolated from a pool of 56 Aedes trivittatus collected between July 27 and September 10, 1964. An alkaline aqueous extract of this agent did not hemagglutinate goose red blood cells. On the third passage, this agent kills suckling mice on the third day post-inoculation.

With the elimination of negative pools nearing completion, efforts are turning toward identification of the 4 isolates. Sensitivity tests will be run with sodium desoxycholate and if indicated, sucrose acetone extracted antigens and hyperimmune serum will be prepared.

REPORT FROM THE UNIVERSITY OF ILLINOIS CENTER FOR ZOONOSES RESEARCH URBANA, ILLINOIS

A collection of 615 blood specimens from residents of McLeansboro, Illinois, and environs was made in the course of field studies associated with the outbreak of St. Louis virus encephalitis in that community. The results of HI and CF test by age group of donor follows.

Age Group of Donor	Number of Donors	Positive SLE	
		Number	Percent
0-5	12	1	8
6-10	92	10	11
11-15	145	8	5
16-20	71	2	3
21-25	17	0	-
26-30	16	0	-
31-35	26	1	4
36-40	42	2	5
41-45	47	2	4
46-50	47	3	6
51-55	38	2	5
56-60	30	1	3
61-65	15	0	-
66-70	8	0	-
71-75	5	0	-
76-80	3	0	-
80 and over	1	0	-
Total	615	32	

In the group of St. Louis positive sera, HI titers ranged from 1:20 to 1:320 and CF antibodies were present in the 1:8 to 1:128 dilutions.

The presence of 32 sera with HI and CF antibody in the group of 615 seems low when one considers the high infection rate in mosquitoes that was reported in Issue #10 of this Information Exchange.

It should be stated that no further cases were reported after September 10-11, the dates when specimens were collected.

In summary, the detection of SL antibodies in bird blood and the isolation of the agent from bird tissues and mosquitoes support the hypothesis that the main cycle at McLeansboro was mosquito-bird-mosquito with only an occasional spill-over to man.

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

Between May and September 1964, neutralizing antibody to Powassan virus was detected in sera from 163 of 454 forest rodents collected in the Powassan-North Bay area of northern Ontario, Canada. Of 340 groundhogs (*Marmota monax*) collected in the townships of Bonfield, East Ferris and Chisholm, 155 had antibody. The prevalence of antibody in juvenile groundhogs increased from 3% during June to about 45% during July and August, but the antibody incidence in adult animals remained relatively stationary at about 50% throughout spring and summer. Performance of hemagglutination inhibition tests immediately after receipt of each week's collection of sera greatly assisted in the concentration of animal collections to localities where high rates of infection were detected amongst rodents.

Powassan virus strains were isolated from 8 of 91 pools of *Ixodes cookei* ticks removed from groundhogs. Each pool contained between 2 and 15 nymphal or adult ticks, and all the ticks from one animals were pooled before inoculation of ground-up suspensions intracerebrally into suckling mice. Virus strains were reisolated from 5 to 6 tick suspensions following inoculations of primary mono-layer cultures of swine kidney cells 1 to 8 weeks subsequently. Virus-positive ticks were collected during May, July and August. Blood fro m 2 of 355 groundhogs yielded strains of Powassan virus after inoculation both of suckling mice and swine kidney tissue cultures. Virus titers per 0.1 ml. ranged from 0.5 to 3.7 log_{10} TCD_{50} in tick suspensions and from 0.7 to 1.2 log_{10} TCD_{50} for blood.

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

POWASSAN VIRUS ISOLATED IN TWO COUNTIES IN NEW YORK STATE

(ELINOR WHITNEY)

Two strains of Powassan virus were isolated from two pools of ticks collected from two Marmota monax trapped at two sites approximately 2 miles apart on Barnhart Island in northern St. Lawrence County. Strain 64-7062 was from a pool of two unidentified ticks from Woodchuck #25 caught July 7 on Barnhart Island proper; strain 64-7562 was from a pool of four Ixodes cookeii from Woodchuck #72 caught July 16 on an "island" separated from the mainland by two locks and a canal, and from the other site by a river. Original isolation of 64-7062 was begun on July 15 and for strain 64-7562 on August 4. Both strains had a similar incubation period. Sodium desoxycholate reduced the LD_{50} titer of $10^{8.9}$ to $<10^2$ per 0.03 ml and ether reduced the titer by 3.5 logs. Powassan immune serum protected mice infected with at least 200 LD_{50} of both strains.

The strains were compared antigenically with the original Powassan virus and the results are shown in Table 1.

Hemagglutination antigens from suckling mouse brains were prepared for each strain by the sucrose acetone method of Clarke and Casals. These antigens were also used for complement-fixation antigens after they had been exposed 4 inches from ultraviolet light on a rocking platform for 30 minutes. The complement-fixation (CF) technic was that of Kent and Fife (Amer. J. Trop. Med. and Hyg. 1963, 12: 103-116). Box titrations were used. Hyperimmune mouse sera were prepared for Powassan and 64-7062 by methods previously described (Whitney, E., Amer. J. Trop. Med. and Hyg. 1963, 12: 417-427). For the CF test, both sera were inactivated at 60°C. for 30 minutes.

Neutralization tests in suckling mice were carried out as follows: Twofold serial dilutions (1:4 - 1:256) of hyperimmune sera were mixed with aliquots of virus diluted to contain an estimated 100 LD_{50}. Virus titrations in normal mouse serum were included in each test to calculate the actual dose used.

The two strains isolated from ticks appear to be identical with the original Powassan virus by all three serologic tests.

Tissue suspensions prepared from blood clot, liver, spleen, kidney, and brain taken from Woodchucks #25 and #72 were inoculated intracerebrally into 1-day-old mice. Seven strains of Powassan virus were recovered. No virus was isolated from the brains of #25 and #72 or the kidney of #72. The seven strains were identified by neutralization tests using two immune sera, one prepared with Powassan virus, the other with 64-7062.

The sera of Woodchucks #25 and #72 showed no demonstrable neutralizing antibodies for Powassan, St. Louis encephalitis, Eastern and Western encephalomyelitis, or Cache Valley viruses. Serum from #25 failed to inhibit hemagglutination of 4 to 8 units of Powassan and St. Louis encephalitis antigens. The serum from #72 had a hemagglutination inhibition titer of 20 in duplicate tests with Powassan antigen. Since Woodchuck #72 was immature, this titer might possibly be residual maternal antibody.

The Rabies Group of the Laboratories for Veterinary Science of this Division isolated in 10- to 12-gram mice an infectious agent from the brain of a gray fox which had been found with choreiform movements and died 48 hours later at Whitney Point in northern Broome County. The fox was received on June 30, 1964. Moribund mice from the 4th mouse brain passage were given to the Arbovirus Group for identification. On intracerebral inoculation of 1-day-old mice, the LD_{50} was $10^{8.9}$ per 0.03 ml. and for the intraperitoneal route was $10^{8.5}$. Two hyperimmune rabbit sera, one prepared with the original Powassan virus, the other with the 1964 tick strain 64-7062, solidly protected 16 mice infected with 40 LD_{50} of the fox strain. No neutralization was evident with sera prepared with the following viruses: MM, herpes simplex, lymphocytic chorio-meningitis, Colorado tick fever, Eastern and Western encephalomyelitis, St. Louis encephalitis, Cache Valley, or Flanders.

Previous serologic surveys (Whitney, E., Amer. J. Trop. Med. and Hyg. 1963, 12: 417-424) established that Group B antibodies were present in 52% of 100 fox and raccoon sera trapped in Schuyler County, a close neighbor of Broome County. This Group B antibody appeared to be closely related to Powassan.

To date, sera from 2 persons, all 11-year-old boy who was a life-time resident of Albany County, and a 51-year-old woman

from Gouveneur, St. Lawrence County, solidly protected mice infected with Powassan virus; these sera also have reproducible low hemagglutination inhibition titers of 10-20 with Powassan antigen.

Table 1

Comparison of two virus isolates with the original Powassan strain

Virus	Complement fixation			Hemagglutination-Inhibition*			Neutralization		
	Mouse sera			4-8 units of antigen Mouse sera			No. LD_{50} used	Mouse sera	
	Pow 10/25/60	64-7062 9/29/64	Nor. 6/24/64	Pow 10/25/60	64-7062 9/29/64	Nor. 6/24/64		Pow 10/25/60	64-70 9/29/
Powassan	128**/32	256/16	< 4	1280	1280	10	124	64***	32
64-7062	256/32	256/32	< 4	320	320	< 10	6500	8	8
64-7562	256/64	256/64	< 4	640	1280	10	200	32	64
Normal	< 4	< 4	< 4						

*Reciprocal of highest dilution of serum inhibiting hemagglutination

**Highest dilution of serum giving 50% hemolysis / Highest dilution of antigen giving 50% hemolysis

***Reciprocal of highest dilution of serum protecting 75% of infected mice

ANTIGENIC COMPARIONS OF FLANDERS VIRUS AND HART PARK VIRUS BY TWO SEROLOGIC METHODS

(ELINOR WHITNEY AND ALBERT ROZ)

Flanders strain 61-7484 and Hart Park strain AR70 were compared by complement-fixation (CF) and neutralization tests. The CF antigens were prepared from infected suckling mouse

brains (smb) using the sucrose acetone method for preparation of hemagglutination antigens of Clarke and Casals (Amer. J. Trop. Med. and Hyg. 1958, 7: 561-573). Immune sera were prepared in mice inoculated intraperitoneally with 0.2 ml. of a 10% smb suspension at weekly intervals. The mice were bled orbitally 7 days after the final inoculation, and the serum was removed and stored frozen at -20°C.

The CF technic of Kent and Fife (Amer. J. Trop. Med. and Hyg. 1963, 12: 103-116) was followed using box titrations (Table 1). All sera were inactivated at 60°C. for 30 minutes. No reactions were obtained with the control serum and antigen. Flanders antigen did not react with the Hart Park immune serum (2 injections) while the Hart Park homologous antigen did. The titer of Hart Park serum (5 injections) was fourfold less with Flanders antigen than with Hart Park antigen, 8 and 32 respectively. The reverse was also true: the titer of Flanders serum with Hart Park antigen was 16 and with the homologous antigen, 64.

Essentially the same findings were obtained in neutralization tests. When Flanders virus was tested with Hart Park serum (5 injections), a titer of 24 was obtained with the heterologous serum and of greater than 256 with the homologous serum (5 injections) - a tenfold difference. When Hart Park virus was tested with Flanders serum, a titer of 152 was obtained with the heterologous serum and a titer of greater than 256 with the homologous serum. Hart Park sera (2 injections) in a 1:4 dilution failed to neutralize Flanders virus; though the tests were irregular, there was some evidence of neutralization of the homologous virus.

These data indicate that Hart Park and Flanders are related but not identical viruses.

Table 1

Antigenic Comparison of Flanders and Hart Park Viruses

Virus	Complement fixation			Neutralization			
	Mouse sera			No. LD_{50} used	Mouse sera		
	Hart Park AR70		Flanders 61-7484		Hart Park AR70		Flanders 61-7484
	2 injections	5 injections	5 injections		2 injections	5 injections	5 injections
Hart Park AR70	4*/16	32/32	16/32	31	**	> 256***	152
Flanders 61-7484	< 2	8/32	64/64	159	< 4	24	> 256

*Reciprocal of highest dilution of serum reacting / Reciprocal of highest dilution of antigen reacting

**Test irregular but showed evidence of some neutralization

***Reciprocal of serum dilution protecting 50% of mice

REPORT FROM THE DEPARTMENT OF TROPICAL PUBLIC HEALTH HARVARD SCHOOL OF PUBLIC HEALTH BOSTON, MASSACHUSETTS

TRANSMISSION OF SINDBIS VIRUS BY *AEDES AEGYTPI* TO A CHICK EMBRYO CELL CULTURE SYSTEM

(JAMES O. MASON
THOMAS E. FROTHINGHAM
ANDREW SPIELMAN
THOMAS H. WELLER)

A chick embryo cell culture containing adenosine triphosphate and covered with an animal-derived membrane was developed. Female *Aedes aegypti* fed upon these cultures readily. After exposure of the cultures to mosquitoes infected with Sindbis virus, plaques appeared within 72 hours and virus was recovered from the plaques.

Materials used in the preparation of the cultures were as follows. Growth medium comprised 9 parts Earle's salt solution containing 0.5 gm lactalbumin hydrolysate, 0.021 gm $NAHCO_3$, and 4 ml 0.05 N tris buffer per 100 ml; and 1 part heated (56°, 30 min.) horse serum. Agar medium consisted of Earle's solution (without $NaHCO_3$) containing Difco Bacto Agar 1.0 gm, gelatin 0.5 gm, Difco yeast extract 0.1 gm, neutral red 0.003 gm, and tris buffer 5 ml per 100 ml. Chick embryo cell suspension was prepared by mixing growth medium containing 10^8 cells per ml with an equal volume of agar medium in a 40° C water bath. Adenosine triphosphate (ATP) solution was prepared by adding 0.31 gm of the disodium salt to 5 ml tris-growth medium. The ATP was dissolved and pH adjusted to 7.5 by the addition of 0.85 to 0.90 ml of 1 N NaOH. This solution was sterilized by filtration. Animal derived membranes (untreated Baudruche) were cut into discs 5.5 cm in diameter and sterilized by exposing both surfaces to ultraviolet light (2537 Å at 5.5 cm, 10 min. each side).

The cultures were prepared in 35 mm diameter plastic Petri dishes. Four ml of agar medium were added to the dish and permitted to solidify. Next, 0.5 ml cell suspension was added; after this layer had hardened, 0.5 ml of a third layer was added. This third layer which was made up of 3 parts agar medium, 4 parts heated horse serum, and 1 part adenosine triphosphate solution, was essential as an attractant and feeding stimulus for mosquitoes.

Before exposing the culture to mosquitoes, the surface was covered by the membrane which was held in place by a cover with a center hole 3 cm in diameter. Mosquitoes readily probed through the membrane and retracted the labial sheath as in feeding on an animal host. The membrane protected the underlying material from microbial contamination. After exposure, the membrane was removed, the culture was covered with an intact lid, inverted, and incubated at 36°C. in humidified air.

REPORT FROM JOAN DANIELS, VIROLOGY SECTION, AND DR. ROBERT J. TONN, TAUNTON FIELD STATION, MASSACHUSETTS PUBLIC HEALTH LABORATORY BOSTON, MASSACHUSETTS

I. Taunton Field Station.

A. Sentinel Flocks:

Sentinel flocks were bled periodically from May 15 until October 28, 1964. The last blood specimens have been sent to the Virus Section, Boston, for testing for EE and WE. Serial bloods from the positive birds will be back-tested to determine time of conversion.

B. Wild Bird Surveys:

Birding was done at sites I and IV and on the Dike. A total of 1,215 birds of 58 species were banded. Of these birds, 1,002 were bled and the blood specimens submitted for virus and antibody study. Birding was also done on Duxbury Beach during September and October. Four hundred and sixty-three birds of 54 species were captured and 263 bloods collected. In addition, 49 bloods were collected during the time of an Audubon Society course meeting at Block Island. This brings a total of 1,314 blood specimens submitted to the Virus Section for testing. The virus results from 1,300 bloods have been received. WE virus was isolated from one robin (8-4-1964 at site I Dike), one catbird (8-11-1964 at site I Dike), and one swamp sparrow (10-14-1964 at site I Dike). Of interest is the appearance

of viremia in the above mentioned swamp sparrow at this relatively late date. The latest previous appearance of WE viremia was in a catbird captured on the Dike 9-20-1963.

The first EE isolate in Massachusetts since 1960 was made from the blood of a swamp sparrow captured at Site I (Dike) on 9-28-1964. Blood samples of this bird were collected 9-4-1963, 9-30-1963 (conversion to WE antibody), and five times in 1964: June, July two times, August, and September.

C. Comparative Antibody Rate Studies:

During the past four years studies have been conducted on recapture of chickadees, swamp sparrows, and catbirds. The testing for antibodies of these birds has provided some interesting field information with regard to persistence of antibody.

A total of 583 samples were collected from these birds in 1963. To date, 456 of these samples have been tested for antibody activity of EE and WE. Of the 456 samples, 65 showed antibody activity. (Thirty-three had WE and thirty-three had EE. One swamp sparrow had antibodies for both viruses.)

A total of 39 swamp sparrows were recaptures, 11 of which were recaptures from previous years. From the 39 recaptured swamp sparrows, a total of 98 bloods were collected. Of these bloods, five had antibodies for WE. However, none of these bloods were from multiple samples of a single bird. Seventeen bloods were positive for EE antibodies: 4 bloods from one bird, 6 bloods from one bird, 2 bloods from one bird, 2 bloods from one bird. A total of 7 birds had antibody for EE.

Twenty-seven catbirds were recaptures. Thirteen of these birds were recaptures from previous years. From the 27 recaptured catbirds, a total of 46 blood samples were collected. Of these bloods, 6 had EE antibody with no multiple samples from one bird, and 8 bloods had WE antibody with 2 bloods from one bird.

A total of 57 chickadees were recaptures. Twenty-four of these were recaptures from previous years. From these 57 recaptured chickadees, a total of 123 bloods were collected. To date, no antibody activity has been recorded from these birds.

D. Reptiles:

Natural antibody for EE has now been found in a total of four spotted turtles. The fourth turtle of this group was one collected in June 1964 from the dike.

Pre-inoculation, 8-day post-inoculation, and 38-day post-inoculation blood specimens were collected from 31 spotted turtles and 3 box turtles used in the 1964-65 overwintering study. Some of these turtles received inoculations of either EE or WE virus and some were kept as controls. No virus was isolated from the pre-inoculation bloods. Results of viremia from the post-inoculation blood specimens or antibody from all specimens have not been received.

E. Mammals:

Ninety-two mammals have been bled since the last report: 50 white-footed mice, 7 red-backed voles, 3 meadow voles, 5 chipmunks, 11 gray squirrels, 10 red squirrels, 5 Norway rats, and 1 cottontail rabbit. Results of 74 of these mammals show them to be negative for viremia. No results of antibody tests have been reported.

F. Arthropods:

Mosquitoes and tabanids were collected this year. A total of 11,963 mosquitoes were collected from the period 6-1-1964 to 10-30-1964, of which 8,803 were frozen into 640 pools. A total of 1,854 tabanids were collected from the period 6-1-1964 to 9-15-1964, of which 1,286 were frozen into 128 pools. None of these pools have been checked for viruses.

The 1963 pools are almost completed. To date, a single pool of *Culiseta melanura* from Pine Swamp has yielded WE virus.

G. Control:

No control program was undertaken this year because of the drought and subsequent low numbers of *C. melanura*.

II. Virus Section.

A. Human Cases:

The first case of human disease in Massachusetts, associated with the finding of WE antibody is reported. The serum neutralized WE virus in 3 separate tests and failed to neutralize EE virus. The latter control would seem to rule out any inadvertent contamination of the specimens with a virucidal substance. The neutralizing antibody was of fairly low titer: 1/7 (50% reduction 1/16). The cause of death was given as meduloblastoma.

REPORT FROM THE YALE ARBOVIRUS RESEARCH UNIT DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH YALE UNIVERSITY SCHOOL OF MEDICINE NEW HAVEN, CONNECTICUT

As of January 1, 1965, the Yale Arbovirus Research Unit (YARU) will have as its new director Dr. Wilbur G. Downs, Professor of Epidemiology. Dr. Downs' staff in New Haven will include Drs. S. Buckley, J. Casals, D. Clarke, J.R. Henderson, N. Karabatsos, R. Speir, T.B. Stim, M. Theiler, R.C. Wallis, and L. Whitman. The old arbovirus unit at Yale is pleased and proud to join forces with the distinguished staff of the Rockefeller Foundation Laboratories previously located in New York, toward the development of a single corrdinated arbovirus program in New Haven.

The host as a determinant in the evolvement of strain variants (Drs. Henderson, Shah, Wallis):

Western equine encephalomyelitis virus strain isolates, designated previously as phase II antigenic variants (J. Immunol., 1964, 93:452), were found to be "contaminated" with the aphasic antigenic type of WEE (14 "contaminated" of 14 tested). During simultaneous multiplication of both antigenic varieties of virus in chick embryo cell cultures, the phase II type was selected and accumulated during a 48-hour period, even when the original inoculum contained 100 times more infectious aphasic virus. The aphasic type, on the other hand, was selected and predominated following

intracerebral inoculation of infant mice with virus mixtures containing 1,000 times more phase II than aphasic virus. *Aedes aegypti* mosquitoes, when infected with such mixtures by feeding, did not select one antigenic type of WEE over another. The selection phenomenon in chick embryo cultures is explained, at least in part, by the differences in the rates at which each antigenic WEE virus type multiplies and/or is released from infected host cells. Preliminary data on the antigenic properties of viruses of several sero-groups would indicate that many serotypes other than WEE are "contaminated" with closely related variants.

Plaque Formation by Dengue Virus Serotypes (Dr. Stim):

Plaques were produced by mouse adapted strains of all recognized dengue serotypes using a modified overlay in cultures of rhesus monkey kidney cells. Overlay modifications included changes in the Mg and Ca ion concentrations and the addition of L-arginine and oxalacetic acid. Endpoints were generally higher in overlaid cultures than in parallel titrations in infant mice. Similar plaquing efficiency could be attained in a stable green monkey kidney line.

Nationwide Survey for Arbovirus Antibodies in Brazilian Military Recruits (Drs. Casals and Henderson):

In collaboration with Dr. J.R. Paul, Director of the WHO Reference Serum Bank at Yale, this laboratory is conducting an antibody survey on approximately 3,000 sera, collected in the summer of 1964, and representing a nationwide sampling of military recruits of Brazil. Sixteen antigens are being used for HI screening. Positive sera by HI are to be retested in tissue culture for neutralizing antibodies. A summary of results after screening a representative number of sera is shown in Tables 1 and 2. In addition, all sera were negative against Maguari (virus related to Cache Valley) and Guaroa antigens.

TABLE 1

Screening for Serogroup B Arbovirus Hemagglutination-inhibiting Antibodies in Sera of Brazilian Military Recruits

	St. Louis	Ilheus	Powasson	Y.F.
No. tested	429	429	395	421
No. pos. - not vaccinated with YF 17D	21	20	11	23
No.pos.-vaccinated with YF 17D	12	11	7	22

TABLE 2

Screening for Serogroups A, C, and Ungrouped Arbovirus Hemagglutination-inhibiting Antibodies in Sera of Brazilian Military Recruits

Serogroup	Serotype	No. tested	No. positive
A	Aura	430	1
	Una	430	3
	Mayaro	430	3
	EEE	430	5
	WEE	430	4
	VEE	430	1
C	Marituba	430	1
	Oriboca	121	1
	Caraparu	51	1
Ungrouped	Tacaiuma	205	2

REPORT FROM DR. WILBUR G. DOWNS
ROCKEFELLER FOUNDATION VIRUS PROGRAM
DIVISION OF MEDICAL AND NATURAL SCIENCES

The Rockefeller Foundation Virus Laboratories in New York have moved to New Haven as of December 14, 1964, to occupy new quarters in the newly finished Laboratories of Epidemiology and Public Health of the Yale University School of Medicine. Yale and Rockefeller Foundation arbovirus activities have been combined under the designation of Yale Arbovirus Research Unit (YARU). New addresses for Drs. Downs, Whitman, Casals, Clarke, Buckley, and Speir are: Yale Arbovirus Research Unit, Laboratories of Epidemiology and Public Health, 60 College Street, New Haven, Connecticut. Dr. Theiler has recently retired from the Rockefeller Foundation and has accepted a joint professorship at Yale in the Departments of Epidemiology and Public Health and in Microbiology. The above address serves for him also.

Dr. Harold Trapido, formerly with the Virus Research Centre, Poona, India, and more recently on study assignment for a year at Oxford, has been assigned to duty in the arbovirus laboratory at the Universidad del Valle, Cali, Colombia. His address there will be Apartado Aereo 6555, Cali, Colombia.

Dr. Jorge Boshell, at present at the Virus Research Centre, Poona, has been assigned to the Belem Virus Laboratory and will be reporting there in mid-1965.

Dr. Jack Woodall of the East African Virus Research Institute, Entebbe, Uganda, has accepted a staff position with the Rockefeller Foundation and has been assigned to the Belem Virus Laboratory, to report there in early 1965.

Dr. Roger Williams of Columbia University School of Public Health, is on a year's sabbatical leave, working with the new arbovirus laboratory in the Department of Bacteriology, University of Ibadan, on medical entomological problems.

Dr. Graham Kemp of the California State Health Department has also recently gone to Ibadan to work with this arbovirus unit as a temporary staff appointee.

Dr. Stanley Ricker, formerly with USPHS-CDC, is working at the Trinidad Regional Virus Laboratory as a temporary staff appointee.

REPORT FROM DRS. DAVID E. DAVIS AND RICHARD L. BEAUDOIN THE PENNSYLVANIA STATE UNIVERSITY UNIVERSITY PARK, PENNSYLVANIA

Studies of potential bird and mosquito reservoirs continue in a tract of forest. In this period (September 15-December 15), 195 birds belonging to 34 species were captured. Blood sera and smears were obtained. Some preliminary analyses of the captures for this year and last year are available. For example, the recapture census method is not suitable for Wood Thrushes, Catbirds, or Vireos. The supposition that net shyness occurred was not supported by an elaborate schedule of alternation of nets. The temperature of each day had no affect on the number of birds captured. The middle two "shelves" of nets capture twice as many birds as the top and bottom combined.

During this period, 52 mosquitoes (_A. vexans_ and _A. triseriatus_) were captured. Larvae of 4 species were obtained, the last on November 9.

At the request of CDC, D.E. Davis and a trainee, Robert McLean, went to Houston to attempt an estimate of the bird population between September 21 and October 31, 1964, following the St. Louis encephalitis epidemic. The result was a rough estimate of about 200,000+ birds. Counts were taken in all areas of the city except the large prairies where only species composition was determined. The predominant resident species were House Sparrow (about 40% of the total), Blue Jay (about 14%), Cardinal (about 5%), Pigeon (about 4-1/2%), Mockingbird (about 4%), Boat-tail Grackle (about 2%), and a Red-bellied Woodpecker (about 2%).

About 62% of the birds were in the residential city blocks, about 20% in wooded areas along the bayous, 8% in city parks, 4% in produce areas, 3% in golf courses and cemetaries, 2% in the downtown blocks, and 1% in miscellaneous flocks and roosts and the zoo. A manuscript is being prepared.

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

I. Electron Microscopy of Dengue Viruses:

Electron photomicrographs of dengue TH-36 (? type 5) virus grown in primary African green monkey kidney tissue culture have been prepared by Dr. Atchison. Numerous round particles, both full and empty when negatively stained, averaging about 30 mμ in size, were seen. The particles had the appearance of many other arboviruses. Dengue types 1, 2, 3, 4, and TH-Sman (? type 6) have now been grown in primary GMK, harvested, and frozen awaiting preparation for electron microscopy studies, along with control uninoculated tissue culture aliquots corresponding to each virus harvest.

II. Comparison of Strains of the California Encephalitis Virus (CEV) Complex:

Comparative studies are progressing with the viruses of the CEV complex, including exotic strains, as mentioned in Infoexchange #10, but are still incomplete due to having received one incorrectly labelled specimen.

A virus sent to us by Dr. Wayne Thompson, Madison, Wisconsin (see Infoexchange #10), was readily shown to belong in the California group by CF, confirming their results. This is the LaCrosse virus isolated by the Wisconsin workers from the brain of a 4-year-old child who died in September 1960. Further comparative CF studies suggest this virus (LaX) is more closely related to the Snowshoe hare (SnH) isolate of Burgdorfer than to the prototype Bfs-283 strain (CEV). Comparative CF studies, including results with trivittatus and the *Aedes infirmatus*, Tampa, Florida, prototype, are shown.

Antigen	Serum				
	CEV	SnH	LaX	Triv.	Florida
CEV	128	32	<4	<4	8
SnH	128	256	16	4	16
LaX	64	128	32	8	16
Trivittatus	16	8	-	64	64
Florida	16	8	-	64	64

LaX is more closely related to CEV and SnH than to either the trivittatus or Florida strains, which are very closely related to each other.

Additional viruses isolated in Florida and shown to belong in the CEV complex by the Florida laboratory have been included in these comparative studies. Three agents, isolated from _Aedes infirmatus_ in 1964, have been shown to be identical to the Florida prototype and quite similar to trivittatus virus. Two agents sent by Dr. Warren Hoffert of the Jacksonville laboratory, isolated from _Aedes taeniorhynchus_ and _Aedes atlanticus-tormentor_ have been shown by CF to be more closely related to the prototype Bfs-283 than to the trivittatus or _A. infirmatus_ isolates. Several other isolates from Florida which behave in a rather similar manner have not yet been included in these cross-comparisons. These agents, in contrast to the _A. infirmatus_ isolates, go readily in weanling mice, achieving titers of 5.4 - 6.8 logs by the i.c. route. Apparently there are at least 2 different viruses present in Florida which belong in the CEV complex.

III. Other Viruses from Florida:

Three strains of Western equine encephalomyelitis (WEE) were sent to this laboratory for confirmation of identification by the Tampa Bay Regional Encephalitis Center. These were isolated from an equine brain, and _Culiseta melanura_ and _Aedes infirmatus_ mosquitoes. Two eastern equine encephalomyelitis viruses, both from _Culiseta melanura_, were also submitted. All identifications were confirmed. These viruses are being further characterized and examined for their potential as antigens for use in the local area. The WEE strains are being compared with the Highlands J strain, supplied to us by Dr. Henderson. Three other viruses from Florida, two isolated from ticks, and one from a cotton rat, are being studied but remain unidentified.

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

Isolation of viruses from Puerto Rican dengue epidemic (Ordonez, Eylar, Cole). In the summer of 1963, during the epidemic of dengue fever in Puerto Rico, field studies were

carried out with a living attenuated type I dengue vaccine in several towns of the eastern part of the island by the Department of Microbiology, University of Maryland Medical School, and the Walter Reed Army Institute of Research. In connection with these studies, other people living in the same areas, most of them not included in the vaccine trial, were studied virologically in order to help establish the causative agent(s) of the epidemic. They were people examined less than 24 hours after the onset of symptoms and whose clinical picture, at that point, resembled that of dengue fever. Their age ranged from 7 years to 64 years. They were distributed in the areas of Fajardo, Ceiba, Naguabo, Humacao, Yabucoa, and Las Piedras. From a total of 54 patients investigated, acute blood samples for virus isolation and serology were obtained in 34 cases. About 3 months later, a convalescent blood sample was obtained from 26 of them. HAI tests carried out using dengue virus antigen types 1-4, TH 36, and TH Sman, showed serological conversion in 23 of 26 tested.

Virus isolation attempts were conducted in Baltimore according to the following protocol. Whole frozen blood was diluted and lines and sublines obtained as shown in Table 1. The diluent used was phosphate buffered saline containing 50% normal inactivated rabbit serum, with penicillin and streptomycin added. The different dilutions were inoculated into suckling mice. For lower passages, mice less than 24 hours of age have been used regularly. Later, 1 to 3 day-old mice have been used. From the original inoculation, brains from about one-fourth of the total animals inoculated were harvested from each dilution, on days 8, 10, 12, and 14 after inoculation. Brains harvested from each dilution were pooled and passed in 3 dilutions and from there on these secondary dilutions were kept separately as sublines. From the second passage (subline), brains were harvested on similar days as before, split in half, and kept frozen separately. Sections from one half were tested by fluorescent dengue antiserum and, when viral antigen was demonstrated, the other corresponding half was passed either individually or pooled with other positive brains from the same dilution. Direct staining technique using mouse anti-dengue type 3 serum labeled with FITC and frozen brain sections 10 micrones in thickness cut in a cryostat were used. The remaining mice from the second passage were challenged with $10^{1.7}$ to $10^{2.5}$ LD_{50}

of a weanling mouse adapted dengue type 4. From fluorescent antibody and challenge results, sub-lines containing the virus were chosen for "blind passage" and adaptation. Mice inoculated with material below the 6th passage showed only occasional and irregular pathogenic signs, such as lethargy and/or weakness or stiffness of the hind legs and instability. Generalized tremor was another sign observed. At about the sixth passage, signs became more prominent and regular in appearance from the 10th to the 14th day after inoculation. Complete hind leg paralysis and convulsions became more prominent after the 6th passage. Auto-interference of some degree was apparent in some cases when different dilutions were inoculated. Four strains are in different stages of workable adaptation at the present time.

Preliminary HI tests using PR-6 B II as antigen, show cross-reaction with sera prepared against D-1 through 4, TH36, TH Sman, TP21 Murray Valley viruses, but not with yellow fever, chikungunya, Bunyamwera, or Apeu viruses, nor with serum prepared against normal mouse brain. At the present time, we can only conclude that this agent is a Group B arbovirus, probably belonging to the dengue sub-group. Definitive tests towards characterization of PR-6 B^{II} and other isolates are underway.

Parallel studies were carried out in an attempt to isolate or to demonstrate virus by tissue culture methods other than the interference technique. The same acute phase serum specimens employed in the initial mouse isolation work were employed as inocula for monolayer cultures of HeLa, KB, BSC-1, and primary chick embryo cells. Both fluid cultures and methyl cellulose overlaid cultures were employed in this study.

Attempts to demonstrate the presence of a plaque forming agent were successful. Seven of the nine acute phase sera, subsequently shown to contain virus by mouse inoculation techniques, formed plaques in at least one of the four cell culture systems employed. Plaques were first noted 10-14 days post-infection and could be demonstrated as late as 21 days after infection of the cell monolayers. Dilution of the initial inoculum resulted in a corresponding decrease in the number of plaques formed in the test cultures. Unfortunately, as was our experience with known strains of dengue viruses, the maximum plaque size seldom exceeded 1 mm in diameter.

In most instances, there was a close correlation between the time of appearance of plaques in tissue cultures and the general appearance of symptoms in the inoculated suckling mice. In both systems, the first evidence of infection occurred 10-14 days after inoculation.

Although varying dilutions of the acute phase sera were inoculated into fluid cultures of HeLa, KB, BSC-1, and primary cultures of chick embryo cells, there was no evidence of a virus induced cytopathic effect during the 21-28 day incubation period. Fluids harvested at 5-day intervals were passed into each of the four cell strains, but without evidence of overt cytopathogenicity.

Table I

Primary Dil. Lines	Secondary Dil. Sub-lines
1:2 A	10^{-1} A I
	10^{-2} A II
	10^{-3} A III
1:20 B	10^{-1} B I
	10^{-2} B II
	10^{-3} B III
1:100 C	10^{-1} C I
	10^{-2} C II
	10^{-3} C III

Liquid nitrogen storage containers for field use. Marshall reported in the October 1964 issue of the Infoexchange the use of portable liquid nitrogen containers for field use. We have been using similar containers (Linde) for the past two years in field trips in the Himalayan Mountains in Gilgit Agency, West Pakistan. These portable containers have completely revolutionized field work in remote areas. We have carried them in jeeps through deserts and over 14,000 ft. mountain passes and have transported them without difficulty by air. Rendevous at an air strip with a courier bringing a fresh 25 liter thermos of liquid nitrogen every 2-3 weeks is all that is required to maintain a constant, reliable low temperature storage of infectious material. This system has been applied successfully to arthropods and animal tissues containing tick-borne viral agents and various species of rickettsiae.

REPORT FROM THE JOHNS HOPKINS C.M.R.T.
DEPARTMENT OF PATHOBIOLOGY, THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF HYGIENE AND PUBLIC HEALTH

Laboratory Infection with Chikungunya Virus--A Case Report.

This is a report of a laboratory infection of an adult Indian male investigator with chikungunya virus.

Exposure: Infection most likely occurred on the afternoon of May 25, 1964, when the investigator was feeding mouse brain virus to a batch of mosquitoes. He had not worked with infective virus for 26 days prior to this date; and while he took care of the mosquitoes infected on May 25, to the best of his knowledge, he was not exposed to the virus in any other manner.

For the feeding of the mosquitoes, a ten per cent mouse brain virus suspension was diluted threefold and was deposited on the top of the cages with a pasteur pipette. After a period of one and one-half to two hours, the remainder was collected with a pasteur pipette for later titration. During the feeding, the mosquito cages were covered with an aluminum pan and were placed in a cubicle

where no one was allowed. After the feeding, the fed mosquitoes were transferred from the virus-contaminated cages to holding cages by means of a mosquito-sucking tube.

The preparation used for feeding was the second infant mouse brain passage virus of the Indian strain 63-266 of chikungunya virus. It titered $10^{8.0}$/ml. in primary hamster kidney tissue culture.

Onset of illness: The onset was on the afternoon of June 2, eight days after the exposure. He was admitted to The Johns Hopkins Hospital on the same evening.

Course of illness: The first symptoms were mild myalgia, headache, and restlessness, followed by fever in the late afternoon. The temperature (rectal or oral) was 102°F.-103°F. on the first and second days of illness, after which it returned to normal. On the third day, it rose up to 99.8°F. for a brief period of time and was thereafter normal. Myalgia and headache were also relieved in 48 hours. There was no lymphadenopathy.

A rash was first noticed at the time of admission to the hospital. It was a fine, blanching, macular-papular generalized rash and was most marked over the trunk and upper arms and less over the legs and face. It disappeared in 48 hours.

The total leucocyte count on admission was 18,000/cu. mm. with a shift to the left; it was recorded at 6,200/cu. mm. the next morning.

The patient was essentially asymptomatic after the third day of illness and made an uneventful recovery.

Virological investigations: The findings are summarized in the accompanying table.

VIROLOGICAL INVESTIGATION OF THE PATIENT

SERUM SPECIMEN NUMBER	TIME AFTER ONSET	VIRUS TITER/ML.		ANTIBODIES TO CHIKUNGUNYA VIRUS	
		IN INFANT MICE	IN HAMSTER KIDNEY CELLS	LOG NEUTRALIZATION INDEX (SERUM DILUTED 1/2)	HI ANTIBODY TITER
64439-1	6 hours*	$10^{6.5}$	$10^{5.2}$		
64439-2	18 hours	$10^{6.7}$	$10^{5.2}$		
64439-3	3 days	N	N	1.0	N
64439-4	4 days	N	N	2.0, 1.7	20, 20
64439-5	15 days			4.0	160
64439-6	78 days			3.2	20

* Titers of similar magnitude from buffy coat and erythrocyte specimens (frozen and thawed).

Virus was isolated from two acute-phase serum specimens collected six and eighteen hours after onset; isolation attempts were successful both in infant mice after intracerebral inoculation and in primary hamster kidney tissue culture. Virus was not isolated in either system from sera of days three and four. The viremia titers are measured in infant mice were as high as $10^{6.7}$/ml. and somewhat lower in hamster kidney cells. The virus isolated from the first serum specimen was tested by neutralization test against a chikungunya mouse antiserum and the patient's fifteen-day serum; both sera neutralized more than four logarithmic units of the virus. The hemagglutination-inhibiting and neutralizing antibody response of the patient is detailed in the table. There is a suggestion that antibodies were present in the serum on the fourth day after onset.

Comments: The exact mode of infection, whether by aerosol or by the oral route or through an unnoticed break in the skin, could not be determined. The low passage level of the virus may have contributed to the ease of infection. After an incubation period of eight days, the patient suffered a mild illness of two to three days duration, characterized by myalgia, headache, and rash. Viremia of fairly high titers was demonstrable in the first 24 hours but was not detectable on day three.

REPORT FROM DR. C. J. GIBBS, JR.

LABORATORY ON SLOW, LATENT, AND TEMPERATE VIRUS INFECTIONS OF CNS
NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS
NIH, BETHESDA, MARYLAND

This report describes a simplified procedure for the production in mice of ascitic fluid containing high concentrations of antibody to Junin virus, the etiological agent of Argentinian hemorrhagic fever in man. The volume of ascitic fluid obtained per mouse and its antibody titer are such as to provide more antibody containing reagent of adequate titer than that provided by serum obtained by usual bleeding out procedures. Furthermore, if not destroyed, the mice continue to produce additional ascitic fluid with useful levels of complement-fixing (CF) antibody over extended periods of time. This technique has facilitated laboratory study of Junin virus, for

although the virus is pathogenic for newborn mice and certain strains of guinea pigs, serial passage and infectivity assays have been encumbered by long incubation periods, poorly defined endpoints, and instability of virus. Tissue culture techniques have not yet replaced those in mice. Attempts to produce an hemagglutinin (HA) with this virus have been unsuccessful in our hands and those of others. The importance of suitable antibody-containing reagents for use in the CF test is thus apparent, in view of the absence of a demonstrable HA antigen and the difficulty of performing with this virus reproducible neutralization (NT) tests _in vivo_ and _in vitro_.

TABLE I

PRODUCTION OF MOUSE IMMUNE ASCITIC FLUID WITH JUNIN VIRUS

No. of Mice	Day of Experiment	Treatment			Harvest of Fluid/Serum*			Homologous CF Reactions (antibody/antigen titer)
					No. of Mice	Total Yield	Average per mouse	
30	1	I	Junin XJ 1:50 + Freund adjuvant**	0.5ml # IP				
29	8	II	Junin XJ 1:50 in PBS***	0.5ml IP				
29	20	III	Junin XJ 1:50 in PBS	0.5ml IP				
29	28	IV	Junin XJ 1:25 + Freund adjuvant	0.5ml IP				
27	34	Tapped for ascitic fluid			9	40cc	3.3cc	160/320
27	42	Tapped for ascitic fluid			27	65cc	>2.4 cc	128/128
		Bled out for blood serum			27	15cc	0.6 cc	512/128

*Total yield is that amount of ascitic fluid and serum collected after centrifugation at 2500 rpm/60 minutes.

**Difco Bacto Freund complete adjuvant control No. 456713

***Phosphate buffered saline pH 7.4

Virus-adjuvant mixture (VAM)

REPORT FROM THE DEPARTMENT OF ENTOMOLOGY AND THE DEPARTMENT OF VIRUS DISEASES

WALTER REED ARMY INSTITUTE OF RESEARCH

Study of the ecology of mosquito-borne viruses were continued on Assateague and Chincoteague Islands, Virginia, during summer 1964 and extended to include a fresh water swamp, the Pocomoke Cypress Swamp, Maryland.

Mosquito collections from the Pocomoke Cypress Swamp and from the coastal areas began in June and continued through mid-October. Virus isolations from these mosquito pools were attempted in 1- to 3-day-old suckling mice by personnel of the Department of Virus Diseases. Twelve isolations were obtained from *Culiseta melanura* collected from the Pocomoke Cypress Swamp (Table 1). Eastern equine encephalomyelitis virus (EEEV) was isolated from two pools of 100 *C. melanura* each captured in the same light trap on 1 September. Western equine encephalomyelitis virus (WEEV) was also isolated from a pool of *C. melanura* collected on 30 September. We believe this to be the first isolation of WEEV from Maryland.

Culiseta melanura was the dominant mosquito species in the swamp, comprising 90% of the total mosquitoes collected from resting boxes and Chamberlain light traps. *Culex salinarius* and *Aedes vexans* were the second and third most commonly collected species. Studies on the bionomics of *C. melanura* in the Pocomoke Cypress Swamp were initiated. No *C. melanura* were taken in traps baited with raccoons or young chickens. Blooded *C. melanura* were tested by the precipitin test for identification of blood-meal sources (Table 3). None of these blood meals were of avian origin. The majority of the mosquitoes tested contained blood from pigs (27% of the total), deer (23%), raccoons (17%), reptiles (17%), and cows (10%). One *C. melanura* had a blood meal from man. Also a preliminary study on the flight range of this mosquito was carried out using fluorescent dye to mark 1,532 wild-caught females of which 94 were recaptured within a 24-hour period. The majority were recaptured within close proximity to the point of release. Some were collected from the farthest light trap, 1/8 mile from the release point 15 hours after release.

In the salt marshes and adjacent woodlands, mosquitoes were captured in Chamberlain light traps and in raccoon-baited traps. *Aedes sollicitans*, *A. taeniorhynchus* and *Culex salinarius* were by far the most commonly collected species of the seven species collected, comprising 33%, 33% and 31% of the total catch, respectively. Results of virus isolation attempts are shown in Table 1.

The Veterinary Science Department, University of Maryland, in collaboration with the Department of Virus Diseases, WRAIR, recently began to collect vertebrates in the Pocomoke Cypress Swamp with emphasis placed upon collection of mammals and reptiles. Animals are captured, bled, marked and released. Virus isolation attempts and serology are being carried out.

(T. Yuill, M. Moussa, M. Collins, E. L. Buescher)

Table 1 Isolates from mosquitoes collected on the Eastern Shore of Maryland and Virginia, 1964

Pool No.	Mosquito Species	Date Collected	Place Collected	Suc. Mouse Incubation	Isolate Identification
M 2448	*Culiseta melanura*	1 Sept.	Pocomoke Cypress Swamp, Md.	2-3 days	EEE
M 2449	*Culiseta melanura*	1 Sept.	Pocomoke Cypress Swamp, Md.	2-3 days	EEE
M 2587	*Culiseta melanura*	30 Sept.	Pocomoke Cypress Swamp, Md.	2-3 days	WEE
M 2410	*Aedes taeniorhynchus*	24 Aug.	Assateague Is., Va.	3-5 days	Bunyamwera Group*
M 2522	*Aedes taeniorhynchus*	27 Aug.- 10 Sept.	Assateague Is., Va.	3-5 days	Bunyamwera Group*
M 2216	*Culiseta melanura*	10-14 Jul.	Pocomoke Cypress Swamp, Md.	6-12 days	Undetermined*
M 2278	*Culiseta melanura*	28 Jul.	Pocomoke Cypress Swamp, Md.	6-12 days	Undetermined*
M 2279	*Culiseta melanura*	28 Jul.	Pocomoke Cypress Swamp, Md.	6-12 days	Undetermined*
M 2281	*Culiseta melanura*	28 Jul.	Pocomoke Cypress Swamp, Md.	6-12 days	Undetermined*
M 2370	*Culiseta melanura*	13 Aug.	Pocomoke Cypress Swamp, Md.	6-12 days	Undetermined*
M 2390	*Culiseta melanura*	13-18 Aug.	Pocomoke Cypress Swamp, Md.	6-12 days	Undetermined*
M 2420	*Culiseta melanura*	25 Aug.	Pocomoke Cypress Swamp, Md.	6-12 days	Undetermined*
M 2467	*Culiseta melanura*	27 Aug.	Pocomoke Cypress Swamp, Md.	6-12 days	Undetermined*
M 2514	*Culiseta melanura*	10 Sept.	Pocomoke Cypress Swamp, Md.	6-12 days	Undetermined*
M 2525	*Anopheles quadrimaculatus*	27 Aug.- 10 Sept.	Assateague Is., Va.	6-12 days	Undetermined*

* Identification in progress

Table 2

VIRUS ISOLATIONS FROM MOSQUITOES COLLECTED DURING 1964

Species	Total specimens	No. pools tested	No. isolations
Pocomoke Cypress Swamp, Md.			
Aedes canadensis	375	9	0
Aedes sollicitans	500	8	0
Aedes taeniorhynchus	325	7	0
Aedes vexans	1025	26	0
Anopheles bradleyi-crucians	450	15	0
Culex salinarius	1525	31	0
Culiseta melanura	28,550	302	12
Uranotaenia sapphirina	75	3	0
Subtotal	32,825	401	12
Assateague & Chincoteague Islands, Va.			
Aedes cantator	25	1	0
Aedes sollicitans	5100	57	0
Aedes taeniorhynchus	4725	60	2
Aedes vexans	75	3	0
Anopheles bradleyi-crucians	75	3	0
Anopheles quadrimaculatus	400	16	1
Culex salinarius	5075	62	0
Subtotal	15,475	202	3
Total	48,300	603	15

Table 3

SOURCE OF BLOODMEALS OF *CULISETA MELANURA* COLLECTED FROM POCOMOKE CYPRESS SWAMP, MD., DURING 1964 AS DETERMINED BY THE PRECIPITIN TEST

Antiserum	Positive reactions	Per cent positive
Pig	13	27
Deer	11	23
Racoon	8	17
Reptile	8	17
Cow	5	10
Dog	1	2
Horse	1	2
Man	1	2
Bird	0	0
Rat	0	0

REPORT FROM DIVISION OF VETERINARY MEDICINE WALTER REED ARMY INSTITUTE OF RESEARCH WASHINGTON, D. C. 20012

(COLONEL R. H. YAGER, MR. A. R. WARNER, AND DR. L. N. BINN)

In cooperation with Dr. H. Dillenberg, Provincial Epidemiologist of the Department of Health of the Province of Saskatchewan, Canada, a large scale study of the respone of human beings to WEE vaccine has been initiated. Sufficient WEE vaccine, produced in chick embryos, partially purified by the technique of Randall, et al., (J. Immunol., 55, 1, 1947) and lyophilized at the Department of Biologics Research, Division of Communicable Diseases and Immunology, WRAIR, was furnished the Provincial Health Department to immunize 1,000 individuals. The first groups were vaccinated in March and April, 1964. Individuals in these groups received three doses of 0.5 ml each, given subcutaneously on days 0, 7, and 21. Pre- and post- immunization serum specimens were collected. The post specimen was taken 14 days after the third dose of vaccine. Representative sera have been examined by the mouse neutralization test using the constant serum-varying virus technique.

The first of two groups immunized consisted of 106 poultry ranchers and their families residing in a part of the province where the disease occurred each year. The second group comprised of 64 employees of the Health Department Laboratory and the Department of Agriculture and Health, all living in the City of Regina. Many of these employees had worked with the virus, both in the laboratory and in the field. Dr. Dillenberg selected sera from each group for initial testing which he felt would give maximum information both as to previous exposure and response to the current immunization program.

Paired serum samples from 36 of the poultry ranchers (Group 1) and 28 of the Provincial employees (Group 2) were tested. The results are shown in Table 1.

TABLE 1

Source	No. Tested	Positive*	Equivocal	Negative**
Group 1	36	11	4	21
Group 2	28	1	1	26

* Difference of 1.2 logs or greater in the paired specimens.

** Difference of 0.5 logs or less in the paired specimens.

Eight of the 36 individuals in Group 1 had pre-immunization antibody. Two were ages 13 and 16 and the balance were 27 years of age or older. Three of the eight had such high levels of pre-immunization antibody that the difference in the paired specimens could not be measured. The other five responded to vaccine by showing a "boost". Thirty individuals in Group 1 were under 20 years of age and six were adults. All of the adults lived in rural areas of the Province and all six had pre-immunization antibody. Group 2 was composed of individuals ranging in age from 19 to 55. The failure of this group to respond to the vaccine is not readily explained. Previous studies have not indicated any difference in the response of different age groups.

Thirteen of the paired specimens have been examined by Porterfield's bead neutralization (BN) and the hemagglutination-inhibition (HI) tests. If the difference in the mouse LD_{50} titers of the sera was greater than 1.0 logs, both the BN and the HI tests detected antibody; if the difference in titer was 1.0 log or less, both the BN and the HI tests were negative.

An additional group of 50 paired sera from Group 1 was tested by the BN test. Pre-immunization antibody was found in six of these individuals, five of whom showed a response to the vaccine. Of the remaining 44, nine converted from negative to positive following vaccination.

Additional sera from groups vaccinated intradermally are to be evaluated. In addition, all sera are being examined for CF antibody by the Department of Health Laboratories, Saskatchewan.

Saskatchewan experienced large outbreaks of WEE in 1941, 1952, and 1963. It is apparent that many of the poultry ranchers had some experience with the virus during one of these outbreaks.

REPORT FROM N. H. WIEBENGA, M.D.
FOR LABORATORY OF TROPICAL VIROLOGY
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
NIH, BETHESDA, MARYLAND

During this reporting period the professional staff included Dr. Paul Woke, Dr. J. S. Rhim and Dr. B. Simizu. Dr. Woke spent much time identifying arthropod specimens and tabulating data he collected in San Joaquin, Bolivia, during the 1964 season. All available data was transmitted to Middle America Research Unit. Drs. Rhim and Simizu are contributing their interest to the problems of virus infections in tissue culture and the identification or production of hemagglutinating antigens.

Chronic Infection of Laboratory Animals

Our last report (InfoExchange #10, page 143) referred to breeding failure of hamsters after infection with Machupo virus. Because of the infectivity of Machupo virus, all animals are isolated in negative pressure isolators. Although the effects of this closed environment were not examined, it seemed possible that chronic virus infection might be related to reduced fertility. After continued efforts, successful breeding and delivery of apparently normal litters was observed. On this occasion, the fertility rate, number of successful pregnancies and litter sizes compared favorably with our past experience. Thus we may conclude that reduction of fertility, if any, was transient in chronically infected hamsters, in whom post-partem viruria was demonstrated.

NIH albino rabbits may now be added to the list of animals demonstrating chronic infection by either Junin or Machupo viruses. In spite of high titering and persistent CF antibody after 4 intravenous doses of virus, viral CF antigen was recovered more than 8 months after the last inoculation of virus. Some rabbits died during this time, presumably from trauma, but the remaining animals were asymptomatic. While animals infected with Tacaribe virus have not been tested for chronic virus excretion, results with Junin and Machupo virus infections suggest the necessity of comparable tests with Tacaribe virus infections. Preliminary evidence also suggests that urinary virus does not consistently demonstrate pathogenicity comparable to that of the virus inoculated. Studies of pathogenicity variation are in progress.

Chloroform Treated Antigens

In confirmation of previous impressions that CHCl[3] inactivated Machupo virus antigens did not provide protection against virus challenge, booster doses were inoculated by the intraperitoneal route. Suckling offspring were not protected against a small challenge dose of homologous virus.

Tissue Culture Studies

Previously reported plaque formation and PFU assay of Machupo virus in continuous rabbit kidney cell cultures (MA-111) was apparently related to the number of transfers ("passages") accumulating during serial propagation of stock cell cultures. As measured by PFU titration of replicate virus samples, sensitivity was reduced in cultures produced after about 70 "passages" of the parent stock cell cultures. Sensitivity of the PFU system was restored by substitution of lower "passages" (9 through 26) cell cultures recovered from frozen stock. Effects of the rate of propagation, or the frequency of transfer, may influence the presumed selection of a cell population resistant to formation of virus plaques, but this was not studied directly. Two ounce bottle cultures of low passage cells were inoculated with a multiplicity ration $\geq$ 1 PFU per cell. These cells were suspended, counted and assayed for plaque forming centers. About one plaque per 200 cells inoculated (0.5 percent) was recovered.

Plaque production in continuous monkey kidney cell cultures (MA-104) was reported by Drs. Tauraso, Wiebenga and Shelokov at the 1964 meeting of ASM. Additional studies of MA-104 cell cultures recovered from frozen stock demonstrated little or no sensitivity by PFU assay of Machupo virus, although both Junin and Tacaribe viruses produced PFU titers comparable to ID_{50} assays in animals.

Tables 1 and 2 summarize results obtained with the Vero Cell line mentioned in the previous exchange. These studies were done in high pssage level (72 to 100) cell cultures and have not been confirmed in cultures recovered from frozen stock. CPE was observed after Junin and Tacaribe virus infections but not after infection by Carvallo strain of Machupo virus. All 3 viruses produced large clear plaques 6 to 10 days after inoculation. Serum neutralization of TC CPD_{50} has been demonstrated. Tacaribe virus growth curve has been demonstrated, and additional studies of plaque neutralization and growth curves with Junin and Machupo viruses are in progress. Studies to document the absence of PPLO or other latent infections of the frozen cell stock are also in progress.

TABLE 1. CPD_{50} AND PFU ASSAYS OF MACHUPO, JUNIN AND TACARIBE VIRUSES IN VERO CELL CULTURES

Virus	Mouse or Hamster ID_{50}[1]	VERO TC[2]	
		CPD_{50}	PFU
Machupo	7.5	no CPE	7.8
Junin	7.0	7.3	7.3
Tacaribe	7.9	7.3	8.0

[1] $1/\log_{10}$ per 0.02 ml.

[2] $1/\log_{10}$ per 0.2 ml.

TABLE 2. CROSS NEUTRALIZATION OF TC CPD_{50} IN VERO CELL CULTURES

Virus	TC CPD_{50} Dose	Rabbit Immune Serum*		
		Machupo	Junin	Tacaribe
Junin	320	<4	50	<4
Tacaribe	320	<4	<4	320

*Serum titer expressed as reciprocal of highest serum dilution that inhibited CPD_{50} of virus dose.

REPORT FROM ENTOMOLOGICAL RESEARCH CENTER FLORIDA STATE BOARD OF HEALTH, VERO BEACH, FLORIDA

Portions of the bait-trap and truck-trap collections of _Culex nigripalpus_ made routinely in the Tampa Bay area in 1963 for SLE virus monitoring were examined for ovarian status, in an effort to learn something of this vector's population structure. During the six-month breeding season, % parous, or parous rate, went through repeated episodes of rise from zero to about 75%, which appeared to coincide with production episodes. The overall parous rate, 33%, was too low for direct application to population studies and suggested a bias toward the nulliparous females in both collecting methods. By contrast, _C. nigripalpus_ collected in Indian River County by the power aspirator developed at Vero Beach to sample whole populations of ground-resting mosquitoes had a parous rate of 72%. This indicates a 90% survival per day and places this mosquito among the longer-lived vector species.

The cylindrical bait trap with conical funnels which is frequently used to collect _Culex tarsalis_ and _C. nigripalpus_ depends, for efficient operation, on a flow of air through the trap. This fact raised the question whether it would be more successful if kept in alignment with the wind. In a special test, two traps controlled by an observer were operated at 0° and at 90° from the wind direction, and a third trap was set at fixed, randomly selected directions. Regardless of the orientation, each trap caught similar numbers of mosquitoes. These results were reassuring in view of the prohibitive expense of a trap which would orientate itself in winds of less than 1 mph. A cheaper way to minimize the influence of wind direction is to revolve the trap very slowly on a vertical axis in a horizontal plane. Such a trap will capture significantly more mosquitoes but not enough more to justify its use as a collecting device.

REPORT FROM DRS. WILLIAM L. POND AND N. JOEL EHRENKRANZ, INFECTIOUS DISEASES DIVISION DEPARTMENT OF MEDICINE UNIVERSITY OF MIAMI SCHOOL OF MEDICINE, MIAMI, FLORIDA

Surveillance of arbovirus activity in Miami at the time of the Caribbean dengue epidemic.

Preliminary accounts of the large dengue epidemic which occurred in Jamaica, Puerto Rico, and other Caribbean

Islands during late 1963 and 1964 have recently become available to the scientific community from a few of the many organizations apparently involved in the study of the epidemic. Although our research unit did not participate in the investigations of these epidemics on the Caribbean Islands, nevertheless, the potential threat of this epidemic to the adjacent United States mainland relates to the possible key importance of the South Florida area and the value of research leading to development and application in South Florida of satisfactory methods for rapidly determining any new activity of arboviruses and the nature of the resulting diseases. Although we have had surprising success in the use of sentinel pigeons since we began using them early in 1963 for detection of activity of SLE (see Infoexchange #9) and although we have formulated plans for the development and evaluation of suitable procedures for determining any possible occurrence in South Florida of activity of yellow fever (and other viruses), this nearby occurrence of extensive epidemics of a Group B arbovirus heretofore unobserved in this hemisphere (dengue type 3?) precipitated an immediate need for rapid determination of the activity of this virus if occurring in South Florida. The population of the Miami area always includes a large number of persons arriving from the Caribbean area, often as visitors or in transit to the north. It was necessary to confirm diagnosis of this disease in man at the earliest possible moment; hopefully before dissemination occurred or soon thereafter.

In association with a Dade County epidemiologist (Dr. John E. Davies) and other members of the local Public Health Service as well as with mosquito control groups, and city management officials a program was initiated whereby all local physicians and para-medical personnel in the Miami area were alerted to the possibility of occurrence of dengue in man in South Florida. They were informed of the probable presenting clinical picture and of measures to be taken to insure immediate serological studies for possible dengue infection. Essentially all persons in the Miami area who were ill during this period with febrile illnesses that could possibly be considered as dengue (including those patients classified as "FUO" "undifferentiated febrile illness", "dengue", and other terms) received special attention. Sera were obtained, extracted, and tested for dengue HI antibodies on the same day in most cases, and in no case later than one day following the specimen collection.

Additional serum specimens were collected day by day until we could state if the disease was dengue or not. Isolation attempts, epidemiological follow-ups, mosquito surveillance, some judiciously applied mosquito eradication, and other obviously related activities were carried out by us and members of the local Public Health agency but will not be detailed here. Together with local health officials, plans were also drawn up for any further action to be taken in the event of any indication that a dengue epidemic was developing in this South Florida area.

We detected only two human cases of dengue disease in the Miami area and these persons had been infected in the Caribbean area. No secondary cases developed here. Although our small unit is committed to arbovirus research programs, we welcomed the opportunity to further evaluate, on a research basis, available methods for rapid and continuous surveillance of arbovirus activity in this particular area. Although we are glad that our services and advice were of value to the community, we do not wish to imply in this news report that we have any interest (or sufficient budget) to take on the responsibilities of any of the public health services in relation to these matters.

VEE Serology of American Indians in South Florida.

We recently learned from scientific publications as well as from news releases, that other investigators have been carrying out arbovirus investigations in South Florida near Miami and reported the isolation of VEE virus from mosquitoes collected in this area. These findings apparently augment their other findings of positive VEE serology of animal and man in the Everglades Swamp, a few miles west of Miami, and of American Indians in South Central Florida.

Our recent studies of 60 human cases of undiagnosed encephalitis, aseptic meningitis, and undifferentiated febrile illness (FUO) occurring in 1961, 1962, 1963, and 1964 in South Florida revealed that VEE was not the cause of infections of these patients. Moreover, these 60 Florida residents had no evidence of other previous VEE infection. However, in continuation of our arbovirus program involving nearby American Indians (see Arbovirus Newsletter #9), their HI antibody titer to VEE virus, among others, was determined.

If one accepts a consistently reproduced HI titer of 1:20 to VEE as a positive result, then over 50% of the Indians tested can be said to have VEE antibodies, some as young as 8 years of age (the youngest tested). Twenty-eight per cent of the Indians have HI titers of 1:40 and over. Periodic bleedings are being made of this population, and they are also being observed for clinical evidence of overt infection in the interim. It is hoped that thereby we may determine the nature of the disease caused by VEE virus whenever it occurs in these people. Further tests are necessary to confirm the observation that VEE viruses are active in our nearby swamps, but not as yet in urban Miami.

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

Dengue

Several hundred acute sera from the 1963 outbreak of dengue in Jamaica have been investigated by different laboratories with the result of no isolation of dengue. Laboratories which have assisted in this aspect of the work are the Trinidad Regional Virus Laboratory, the Rockefeller Foundation laboratories in New York, Yale University, University of Pittsburgh, Rutgers University, and the Communicable Disease Center. Attempts are still being made by us to isolate the virus, and it is hoped that we will be successful in due course.

Within the last quarter, very few clinical cases of pyrexia were referred to the virus laboratory. No virus was isolated from any of these, and in serology they were all negative. Six clinical cases of encephalitis were reported. Thus far, none of these proved to be of arbovirus type.

Survey Sera

A total of 264 equine sera submitted by the Ministry of Agriculture and Lands were tested for HAI antibodies to EEE. One serum gave a titer of 1:80. This animal came from Manchester, one of the central parishes. There is no history of vaccination. It will be recalled that in November of

1962, Jamaica experienced an epizootic of EEE in St. Thomas, one of the eastern parishes.

The equine sera were also tested against SLE and 31 (11.7%) gave HAI titer of 1:40 or greater.

Ornithology

Since the last report, the field program in ornithology as directed towards EEE, has been intensified. Dr. Brooke Worth of the Trinidad Regional Virus Laboratory has organized a banding program in two localities--one at Folly near Port Antonio, and the other at Amity Hall, one of the areas which experienced EEE during the outbreak in 1962. The program is aimed at studying the movements of migrants in the area, and also at determining whether there is a cycle of EEE still operating in the area. The birds are bled initially at the time of banding. If, after a period of four weeks, these birds are again caught, they are bled. Since the program started, 18 paired sera were tested for EEE antibody. They were all negative.

Ticks

Work has started towards investigating ticks in Jamaica for any possible role they play in virus transmission. Several thousand specimens of Boophilus spp. and Amblyoma spp. have been collected, and these will be examined in due course.

An analysis of eastern equine encephalomyelitis survey in Jamaica

A summary of serological survey for EEE HAI antibody following the outbreak of EEE in Jamaica in November 1962 is submitted in Table 1.

TYPE SERA	1962 (Nov.19 - Dec.31)			1963(Jan. - Dec.)			1964(Jan. - Dec.)		
	≠ tested	Pos.	%Pos.	≠ tested	Pos.	%Pos.	≠ tested	Pos.	%Pos.
Human	242	6	2.5	56	5	8.9	125	0	0
Equine	698	101	14.5	265	56	21.1	268	1	0.4
Avian	-	-	-	32	1	3.1	126	1	0.8
Rodents	-	-	-	-	-	-	89	2	2.2

Human Sera. The positive human sera for 1962 and 1963 came from individuals who received EEE vaccine during the course of the epidemic. However, one individual who recovered from EEE in 1962 gave a 1:80 titer.

Equine Sera. Concerning the equine positive sera for 1962, 89 came from animals in St. Thomas parish where the outbreak occurred. The remaining positive sera for 1962 and probably all of those for 1963 came from vaccinated animals. The titer in most cases was 1:10 - 1:40. The single positive serum for 1964 came from Manchester, one of the central parishes, about 90 miles away from the outbreak area. It is not known whether this animal had been vaccinated.

Avian Sera. During 1962, all avian sera were tested only in neutralization test. Out of 170 sera tested, there were nine positive (5.3%). In 1963, a total of 202 bird sera were tested in NT; three of these were positive (1.5%). One serum which was positive in HAI was confirmed by NT. All these birds came from the parish of St. Thomas and within the EEE area. The species of birds which showed neutralizing antibody to EEE are listed below:

Dendroica dominica	(2)	Contopus caribaeus	(1)
☆ Coereba flaveola	(1)	☆ Dendroica caerulescens	(1)
☆ Tiaris olivacea	(1)	Dendroica tigrina	(1)
Spindalis zena	(1)	☆ Dendroica coronata	(1)
Euneornis campestris	(1)	Gallus domesticus	(2)

☆ Partial protection.

Rodent Sera. The investigation of rodent sera for EEE antibody started in 1964. The species of rodents collected were Herpestes javanicus auropunctatus, Rattus rattus, and Rattus norvegicus. Out of 89 sera tested in HAI, there were two positives. Both of these sera came from Herpestes javanicus auropunctatus (Mongoose). One of the animals was caught in St. Thomas and the other in Portland, an adjoining parish to St. Thomas. Both sera were positive also in HAI test to St. Louis encephalitis antigen.

Latex agglutination test for diagnosis of arbovirus infections.

The drop modification of latex agglutination test was used for the diagnosis of some arbovirus infections. The test is simple to perform and easy to read. It gives results comparable with the more complicated serological procedures and is more practicable for routine laboratory use. Some factors were studied which influenced the results of latex agglutination test used for the detection of arbovirus antibodies. Among others, the antigen concentration and the pH of buffer solution seem to be the most important as they influence the titers obtained. The test performed under rigidly standardized conditions, gives reproducible results which compare favorably with hemagglutination inhibition test. The latex agglutination test can be used also for the detection of some arbovirus antibodies for which hemagglutination techniques present some difficulties, as hemagglutinins are not readily prepared. In our experiments, mouse brain antigens prepared according to sucrose-acetone method were used for the coating of latex particles of uniform size (0.81 mμ).

REPORT FROM THE TRINIDAD REGIONAL VIRUS LABORATORY

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

Studies in Bush Bush Forest

In previous issues of this exchange, we mentioned the serious decline in rodent population density. The population reached the point of virtual extinction during the second half of 1963. During 1964, the rodent population has not recovered. Continuous observation on virus activity in Bush Bush showed that the rodent associated agents VEE, Caraparu, Guama, and Catu were in 1964 either no longer present or active below detectable levels. Bimiti virus, which also had demonstrated a strong association with rodents, was the only virus of this category which was isolated from Bush Bush material in 1964 (twice from sentinel mice, 4 times from *Culex* sp. #9). Vector mosquitoes were abundantly present during this year. Laboratory studies with Guama group agents in fully susceptible and cross-immunized animals indicated that under conditions of host scarcity, Catu virus, rather than Bimiti, had the best chances for continued existence. The field data were therefore believed to

indicate that an additional host for Bimiti virus, but not for the other four agents mentioned, was available in Bush Bush. A search for this hypothetical host is presently underway.

Mosquito colonization

Late in 1963, Dr. Mitsuo Takahashi undertook to establish a laboratory colony of one of our important vectors of virus, *Culex* (*Melanoconion*) sp. #9. The life cycle roughly occupies three weeks at ambient (outside insectary) temperatures (77-81°F). By the end of November 1964, the colony had passed through about 17 generations. As a precautionary measure, a satellite colony is maintained in another cubicle.

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

During the 3-month period from June to September, an ornithological study in cooperation with Dr. Philip S. Humphrey of the Smithsonian Institution was undertaken in and around Belem. Birds were captured both in the forest and in fields. Nine hundred seventy-nine bloods were inoculated i.c. in baby mice and 761 plasmas were tested for HI antibody using the microtiter technique.

Initial HI antibody studies indicated activity in forest birds of viruses of groups A, B, and Turlock. All three were subsequently isolated--3 strains of SLE, 1 of WEE, and 2 of Turlock virus. All viruses isolated came from forest birds, none from open field birds. In addition, significant HI reactions in forest birds (see table) were detected for EEE, Jurona, and Itaporanga viruses. EEE was subsequently isolated in sentinel mice in September, and Itaporanga had been isolated from sentinel mice, mosquitoes, and arboreal marsupials earlier in the year. EEE, Jurona, and Itaporanga were not isolated, however, from the birds.

HI studies of WEE and Turlock in rodents and marsupials living in Utinga forest during this same period were completely negative. Rodents had HI antibody for SLE but in every case were positive for Bussuquara also. The evidence would indicate that WEE, Turlock, and SLE were active in forest birds and probably not in forest rodents and marsupials in Utinga forest this year.

SURVEY OF BIRD PLASMAS, BELEM, BRAZIL, 1964

	Number Plasmas	PER CENT POSITIVE HI RESULTS									
		Group A	Group B	Caraparu	Jurona§	Turlock	Tacaiuma	Maguari	Guaroa	Candiru §	Itaporanga
FOREST AREAS											
IAN	463	8.4	6.9	0.4	10.6	3.2	1.3	0	0.2	0.1	2.8
Utinga	52	15.4	11.5	1.9	0	3.8	0	0	0	0	1.9
FazendaVelha	19	5	0	0	-	0	0	0	0	0	0
BLACK VULTURES (FOREST NESTING)											
IAN	17	6	41	0	-	6	0	0	0	0	0
Icoaraci	10	0	30	0	-	10	0	0	0	0	0
FIELD AREAS											
IAN Pump and Dendê	20	0	0	0	0	0	0	0	0	0	0
FazendaVelha	151	1.3	0	0	3.4	0.7	0	0	0	1.1	0
VSF School	29	7	0	0	0	0	0	0	0	0	0

§ Not all plasmas tested with Jurona and Candirú antigens.

Conversely, isolation and HI antibody evidence has been found during this period in mammals of Utinga and IAN for activity of Mucambo, Bussuquara, Murutucu, Itaqui, Guama, and Moju viruses. Significant evidence for activity of these viruses in birds was not found.

REPORT FROM DR. CARLOS CAMPILLO SAINZ AND DR. JULIO DE MUCHA MACIAS INSTITUTO NACIONAL DE VIROLOGIA, MEXICO, D.F.

Serological studies conducted in July 1962 in the state of Campeche provided, for the first time in Mexico, evidence of Venezuelan equine encephalomyelitis (VEE) human infection (Infoexchange #7). This finding called for further field studies in other areas of the country. Attempts to isolate VEE virus ended in its recovery from a sentinel hamster exposed in the forest of Sontecomapan, Ver. (Infoexchange #10). Human serological surveys were made in the southeastern states, namely Quintana Roo, Tabasco, Veracruz, and Yucatan. The present report deals with the latter point.

Seven hundred and seventy blood samples were collected from apparently healthy individuals of both sexes, ranging from 1 to 75 years of age. A group from Tlacotalpan, Ver., was bled every year since 1961, yielding a total of 308 blood samples. Four hundred and sixty-two individuals from the other localities were bled only once. All the sera were tested by HI using VEE antigen (FD strain). Positive sera were later studied for NIT. A total of 24 sera (3.1%) out of 770 were positive by HIT and NIT to VEE. Their distribution for states is as follows: (No. Positive/No. Studied) Quintana Roo 8/128 (6.2%); Tabasco 3/40 (7.5%); Tlacotalpan, Ver. 7/308 (2.2%); and Yucatan 6/294 (2.0%). In Tlacotalpan, a serological conversion was found in a 10-year-old boy whose serum was negative in 1961 and positive by HIT and NIT in 1963. The infection occurred without clinical manifestations.

These results show an important activity of VEE virus among the human population of the southeast of Mexico.

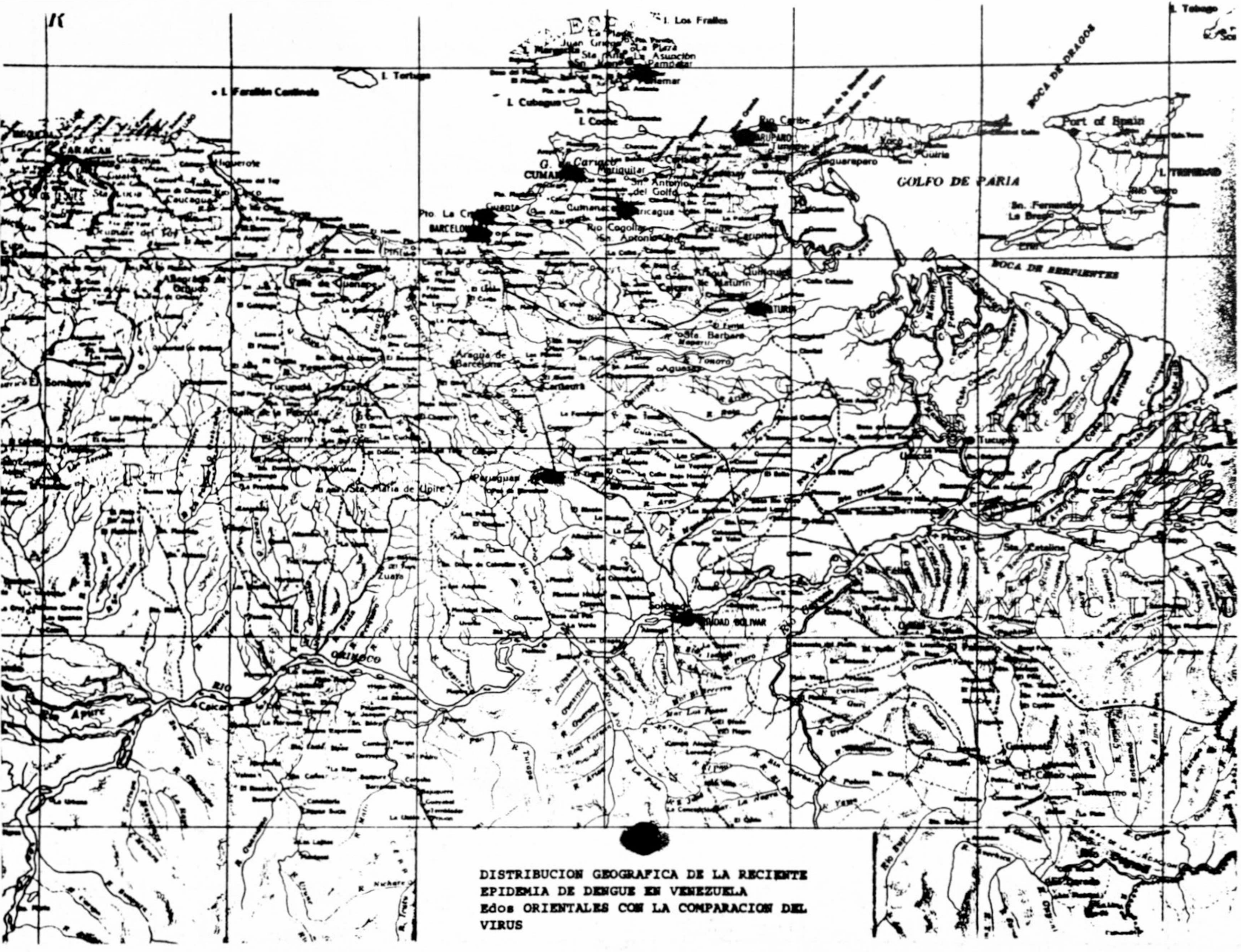
REPORT FROM DR. A.L. BRICENO ROSSI
INSTITUTO NACIONAL DE HIGIENE, CARACAS, VENEZUELA

The Arbovirus Laboratory of our Virus Section has identified cases of VEE in another two states of Venezuela, namely Sucre and Monagas, through isolation of virus in diseased people. A survey is also being carried out among healthy people, in which a total of 5,674 sera from rural areas in 16 states and Territorio Amazonas were processed by HI test. Analyzing survey results, we found the total absence of HI antibody in the rural population of the State of Nueva Esparta, which is an island located between latitude 11th North and 64th South. There are horses and donkeys on the island, but in rather limited numbers; which makes us presume that there is no primary reservoir in this area, and also that birds and fowl do not play any role as reservoirs of VEE virus. We have also shown the absence in 138 birds of either virus or VEE antibody. On the other hand, in a rustic place--an Indian village in Territorio Delta Amacuro--we found that the tribe living there and other natives who did not emigrate from that region show over 1-160, 1-320, and 1-640 antibody in HI test. No horses or donkeys have ever come to this area, which makes us think of the presence of another reservoir in the proximity of this Indian village, which we will study in the near future.

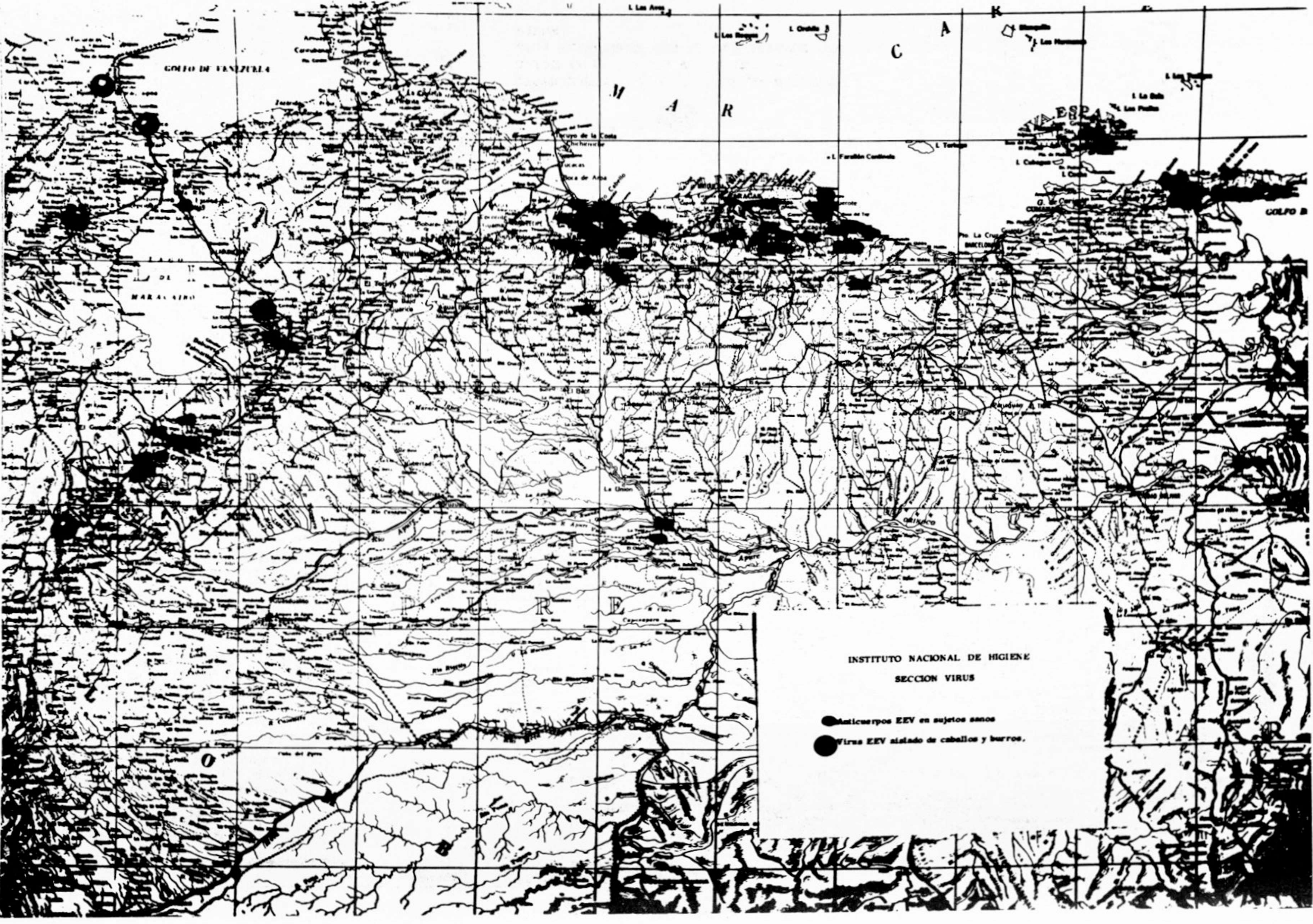
The enclosed map shows that in the serologic survey of healthy persons, wherever we found antibody by HI test, we also found horses and particularly donkeys infected with encephalitis, and VEE virus was isolated. Our conclusions included, therefore, the donkey and horse reservoir as the source of infection of human virosis borne by various mosquitoes, particularly the *A. taeniorhynchus*.

We have had a dengue epidemic in eastern states such as Sucre, Monagas, and Anzoategui (see picture), and recently in the central part of the country, Santa Teresa in the State of Miranda.

On the basis of the routine of inoculating sera collected during the first 3 days of the infection process from cases of dengue, we think that the virus of this epidemic is not infecting suckling mice. In the HI test with dengue 2 antigen, we challenged serum I from the infection period and serum II from the convalescence period. A progressive increase of antibody in convalescents, sometimes as high as 1-5000 or more HI titer compared to 1-20 in serum I was found.



DISTRIBUCION GEOGRAFICA DE LA RECIENTE EPIDEMIA DE DENGUE EN VENEZUELA Edos ORIENTALES CON LA COMPARACION DEL VIRUS


GOLFO DE VENEZUELA
INSTITUTO NACIONAL DE HIGIENE
SECCION VIRUS
nticuerpos EEV en sujetos sanos
irus EEV aislado de caballos y burros.

In our efforts to confirm dengue viruses, we carried out inhibition tests using as host cells the tissue cultures of stable KB (Eagle) cells in which the virus grows without CPE lesions. Such cells were originally received from Microbiological Associates of Bethesda, Maryland. As interfering virus, we used serum from cases of dengue in epidemic areas collected during the infection period, in which virus particles should exist. Two-tenths ml were inoculated per tube, rinsed two hours after inoculation at 37°C, and kept for 48 hours at 37°C in maintenance medium, adding 100-200 TCD_{50} of poliovirus I (Mahoney) for 48 hours at 37°C, then reading the results. With this technic, we found frequent interference of polio I, which was compared to polio I control tubes in the same KB cells and to non-inoculated KB cell control tubes.

This appears to us as a simple method for speedy viral diagnosis of dengue without having to wait for a number of days as in the case of the HI test which requires sera collected from convalescents after 10 days.

In order to distinguish VEE, which can appear--as happened to us in Carupano--in the form of a dual EEV and dengue epidemic, they are inoculated simultaneously into suckling mice which fall ill within 24 to 48 hours, whereas not so with dengue. If it did, the incubation period would be longer than 7 days.

The dengue epidemic reached over 10,000 clinical cases in a saddle-back curve epidemic, with frequent eruptions and some relapses.

REPORT FROM DR. G.H. BERGOLD, HEAD, VIROLOGY DEPARTMENT INSTITUTO VENEZOLANO DE INVESTIGATIONES CIENTIFICAS (IVIC) CARACAS, VENEZUELA

<u>VEE outbreaks</u>. Five VEE isolations were accomplished from serum samples taken from 8 patients in Caripe and surroundings (Estado Monagas) between August 2 and September 19, 1964. This indicates that VEE is still active in this area, which is 800-1000 m above sea level.

<u>"Dengue"</u>. An epidemic began in June in Carupano and surroundings (Estado Sucre) which effected subsequently

INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS

DEPARTAMENTO DE VIROLOGIA

Insects Captured In Venezuela Between July 23 and September 21, 1964

Localities:	Carúpano	Güiria etc.+	Caripe	Aricagua	Porlamar++	Total
Date:	July 23 Aug. 1	July 23 Aug. 2	Sept.19	Sept. 20	Sept. 21	
Aedes aegypti	116	41	0	8	35	200
Aedes scapularis	0	25	0	0	0	25
Aedes taeniorhynchus	4	1220	0	0	0	1,224
Aedes ioliota	0	4	0	0	0	4
Aedes (Soperia) sp.	0	1	2	0	0	3
Anopheles aquasalis	0	1193	0	0	0	1,193
Anopheles pseudopunctipennis	0	2	0	0	0	2
Anopheles strodei	0	4	0	0	0	4
Culex pipiens fatigans	90	562	15	5	12	684
Culex sp.	0	7	0	0	0	7
Deinocerites cancer	0	658	0	0	0	658
Deinocerites spanius	0	3	0	0	0	3
Haemagogus equinus	0	25	0	0	0	25
Haemagogus sp.	0	16	0	0	0	16
Psorophora ferox	0	1	0	0	0	1
Uranotaenia ditaenionota	0	1	0	0	0	1
Wyeomyia sp.	0	349	0	0	0	349
Culicoides sp.	0	4723	0	0	0	4,723
TOTAL	210	8835	15	13	47	9,138

No virus was isolated from these insects

\+ Carenero, Pto. Hierro, Macuro, Tunapui and Sta. María.

++ Isla Margarita.

several tens of thousands of people. Symptoms were fever up to 40.5° C for 3-4 days, followed by rapid recovery. There was no hospitalization and no mortality. Three to four days after onset of fever, there was rash in up to 80% of the cases, sometimes accompanied by vomiting, severe head and body ache, and irritation of the eyes. During two visits (July 23-August 1 and September 19-21, Bergold, Suarez, and Work), there were about 9000 mosquitoes collected, as summarized in the table on the preceding page. Many mosquitoes (*Aedes aegypti*) were collected in the bedrooms of patients, but no virus could be isolated either from mosquitoes, patients sera (with high fever) or by exposing baby mice in the bedrooms. However, three sera produced "dengue"-like plaques in MA 104 and BHK 21 cells. In spite of great effort and several passages, the virus could either not be passed or produced no plaques in any passage. Baby mice showed no symptoms whatever with patients' sera or after several passages. Investigations for antibodies in sera of recovered patients and domestic and wild animals are presently being carried out.

The new virus building is almost completed and will be occupied in February 1965.

REPORT FROM DRS. KATSUJI NAGAI AND SUNTHORN SRIHONGSE GORGAS MEMORIAL LABORATORY, PANAMA

Plaque Formation of Arboviruses in Hamster Kidney Cells Under Single Agar Overlay

With the modified composition of agar overlay medium, hamster kidney cell monolayers can be maintained under single agar overlay at least for a period of one week at 37°C.

Venezuelan equine encephalitis virus plaques, about 0.2-0.6 cm, hazy, round, and ellipsoid with sharp boundaries were obtained within 3-4 days by this technique, while eastern equine encephalitis virus produced somewhat smaller plaques on the 6th day after agar overlay.

The constituents of the medium used for overlay in each 100 ml was as follows: 25 ml of 2% Lactalbumin hydrolysate in

4X Hank's BSS, 4 ml fetal bovine serum, 1 ml antibiotics (final concentration = 100 U penicillin, 40 mg streptomycin, 10 U polyminin, and 25U mystatin per ml), 25 ml of 1% Tris, 2 ml of 1:1,000 filtered neutral red, 0.1% of 1M Mg Cl2, 15.4 ml of double distilled water and 50 ml of 3% Difco Bacto-agar.

Experiments have shown that 2% of 1:1000 neutral red is optimal whereas a concentration higher than 4% readily destroys the cells. Other experiments also indicated that these cells could not survive if the agar medium contained more than 0.1% of 1M MgCl2. The optimal time for virus adsorption was 2 hours at 37°C.

This technique overcomes the more complex double agar overlay method usually used for the hamster kidney cells in plaque assays. Other arboviruses are being studied by this technique.

REPORT FROM THE MIDDLE AMERICA RESEARCH UNIT (NIAID) PANAMA

Studies on Hemorrhagic Fever in Bolivia

Epidemic surveillance has been maintained in San Joaquin. No further cases have been reported among residents of the town since June 28 of this year. Rodent control using traps has also continued, and spot checking indicates population of *Calomys callosus* is being kept at very low levels. During September, three clinically suspect cases were reported from the area of San Ramon, a town located about 15 miles south of San Joaquin on the Machupo River. Rodent control measures were initiated in this town, and no further cases have been reported.

Laboratory reared *Calomys callosus* have been infected with Machupo virus, and the pattern of chronic infection previously reported for hamsters was reproduced. Urine samples taken after the 12th post-inoculation day regularly contain from 10^{2}-$10^{4.5}$ HLD_{50}/ml of virus. About 6 weeks after inoculation, individual animals displayed simultaneous viremia, viruria, and had circulating neutralizing antibodies. A group of 17 healthy *Calomys* captured from 13 houses in San Joaquin during a 7-day interval in May 1964 yielded 13 Machupo virus isolations from spleen and/or

kidney tissue. Animals from 10 of the 13 houses were positive. Five of nine urine samples obtained from these animals also yielded virus.

These findings, together with previously reported data and the continuing negative attempts to isolate the agent from various arthropods, including mosquitoes, add further support to the hypothesis that the disease was transmitted directly from Calomys to humans, most likely by urinary contamination of food, water, or air.

REPORT FROM CATEDRA DE MICROBIOLOGIA Y PARISITOLOGIA FACULTAD DE MEDICINA UNIVERSIDAD DE BUENOS AIRES, ARGENTINA

TO: PARTICIPANTS, AMERICAN HEMORRHAGIC FEVER INFORMATION EXCHANGE

VIREMIA IN HUMAN CASES OF REPERTUIAN HEMORRHAGIC FEVER

(M. C. BOXACA, L. B. DE GUERRERO, A. S. PARODI H. RUGIERE Y S. GONZALEZ CAPPA.)

Viremia was studied in 23 patients with A.H.F. The virus was isolated by inoculation of daily blood samples from those patients in guinea pigs by i.p. route.

In order to confirm the presence of virus, organs from the animals dead during the observation period were inoculated to guinea pigs and suckling mice.

As the blood samples were from patients with complement fixing reaction positive or with clinical diagnosis of A.H.F., we considered the typical signs in guinea pigs and suckling mice to be enough for virus identification.

The viremia may appear as early as the 2nd day of illness and last until the 12th day. It is more frequently observed between the 3rd and the 8th day of illness.

The viremia and hypertemia are almost coincidental. The leukopenia and marked reduction of platelets accompany those alterations but can reach their normal values, a few days after the viremia has disappeared.

This is the general behavior; some exceptions can be observed.

TABLE I

VIREMIA IN 23 CASES OF HUMAN A.H.F.

CASE	DAY OF ILLNESS													COMPLEMENT FIXATION
	1	2	3	4	5	6	7	8	9	10	11	12	13	
1.A.A.		0	+	+	+	+	0	+		+	+‡			
2.B.E.				+	+	+	+							
3.B.M.			0	Op	0	0	0	0						Pos.
4.B.P.		+p	+	p	+	+	0							Pos.
5.B.A.						+	0	0	0					Pos.
6.C.H.				+	0	+		0	0	0				
7.C.A.	0	Op	0	+	+	0	+							Pos.
8.D.E.						+	+	+						Pos.
9.Ch.O				Op	0	+	0	0						Pos.
10.Ch.D				+	0	+	+	0	0					Pos.
11.DiAL			+	0	0		+	+p		0	+			Pos.
12.F.R.							+	+	0					
13.G.R.				+	+	+	0							Pos.
14.L.R.			+	+	+	+	+	+	+	+	+	+		Pos.
15.Lz.R.			0	Op	0	0	0	+	0					Neg.
16.M.F.				0	+		0	+	0		0	0		Pos.
17.M.D.				+	+	+	+	+			0			Pos.
18.M.J.					+	+	+	+	+		0	0		Pos.
19.M.L.			+	+	+	+								Pos.
20.O.N.				+p	0	0	0	0	0	0				Pos.
21.R.R.				+	Op	+p	0		+	0		0	0	Pos.
22.S.A.		+	+	+	0									Pos.
23.Z.R.			+	+p	+	+p	0	+						

‡ = death
+ = isolation of virus positive
0 = isolation of virus negative
p = convalecent plasma
Pos= complement fixation positive
Neg= complement fixation negative

EXPERIMENTAL HEMORRHAGIC FEVER IN GUINEA PIGS: CONTAMINATION AND VIRUS EXCRETION

(L. B. DE GUERRERO, M. C. BOXACA AND A. S. PARODI)

In this paper are studied the possibility of contamination between infected and normal guinea pigs, the infection of guinea pigs by airborne route and the elimination of virus by urine and stools of infected animals.

Normal guinea pigs were exposed to infection by placing them in the same cage and in neighbouring ones to the cages of inoculated guinea pigs (16 strains of Junin virus were employed).

The 100% of inoculated guinea pigs died with the typical signs of AHF. Only the 11.3% of normal guinea pigs placed in the same cage were infected. This means that the 31% of the strains employed contaminated the normal animals. None of the normal animals placed in neighbouring cages died.

By inoculating guinea pigs with daily urine samples taken from guinea pigs infected with Junin virus (strain XJ) was found that the virus appears in urine from the 7th day after the inoculation.

The virus was not isolated from stools of infected animals. Four guinea pigs were infected by nebulization with a suspension of the virus. The animals died with the typical signs of AHF.

TABLE I - CONTAMINATION OF NORMAL GUINEA PIGS BY CLOSE CONTACT WITH ANIMALS INOCULATED WITH DIFFERENT STRAINS OF JUNIN VIRUS

STRAINS	INOCULATED ANIMALS	NORMAL ANIMALS	
		TOTAL NUMBER	NUMBER OF INFECTIONS
XJ stock 9	3	4	1
XJ stock 11	4	4	-
XJ stock 11	2	2	-
XJ stock 11	2	2	-
XJ stock 11	2	2	-
AA	6	2	-
BA	2	2	1
BT	2	2	-
BP	4	2	-
ChD	2	2	-
DE	5	2	-
DiAE	2	2	1
FR	2	2	-
GR	4	2	1
LR	2	2	-
MD	2	2	-
MJ	2	2	-
RR	1	2	1
SA	1	2	-
ZR	6	2	-
TOTALS	56	44	5

TABLE II - ASSAY FOR CONTAMINATION BETWEEN GUINEA PIGS INOCULATED WITH JUNIN VIRUS (STRAINS XJ) AND NORMALS ONES PLACED IN NEIGHBORING CAGES

BOX	INOCULATED GUINEA PIGS	NORMAL GUINEA PIGS	
	NUMBER	NUMBER	NO OF INFECTIONS
1	2	4	-
2	2	4	-
3	2	3	-

TABLE III - ISOLATION OF VIRUS FROM URINE AND STOOLS OF GUINEA PIGS INOCULATED WITH JUNIN VIRUS (STRAINS XJ)

SOURCE	NUMBER OF SAMPLES	NUMBER OF POSITIVES
Urine	6	6
Stools	6	-

TABLE IV

DAILY ISOLATION OF JUNIN VIRUS (STRAINS XJ) FROM URINE OF INOCULATED ANIMALS

DAYS AFTER THE INOCULATION	NUMBER OF INOCULATED ANIMALS	ISOLATION OF VIRUS ∓
1	2	0/2
2	2	0/2
3	2	0/2
4	2	0/2
5	2	0/2
6	2	0/2
7	2	2/2
8	2	2/2
9	2	2/2
10	2	2/2
11	2	2/2
12	2	2/2

∓ - Number of positives / Number of inoculated animals

(DRS. A. S. PARODI, S. GRINSTEIN Y CELIA E. COTO)

The effect of E. coli endotoxin on guinea pigs inoculated with Junin virus was studied. It was intended to get some information about the mechanism of the hemorrhagic signs observed in these animals.

First the susceptibility of normal and inoculated guinea pigs to different doses of lipopolisaccaride was studied. More than 50% of guinea pigs inoculated with 10^3 LD_{50} of Junin virus died when injected after 6 days with 25 γ of lipopolisaccaride; the normal animals need 200 - 400 γ (Table I).

This susceptibility starts 5 days after the inoculation with Junin virus.

TABLE I

Inoculated endotoxin i.p.	Normal guinea pigs (x)	guinea pigs inoculated with 25 γ of endotoxin 6 days after 10^3 LD_{50} of Junín virus (x)
25	-	3/4
50	0/5	4/4
100	0/4	4/4
200	2/5	4/4
400	2/4	-

TABLE II

Days after the inoculation of 10^3 LD_{50} of Junín virus	Animals inoculated with 100 γ of endotoxin
-1	0/10 (x)
1	0/5
3	0/5
5	4/5
6	19/20 (xx)
7	4/5
10	5/5

x $\dfrac{\text{N° of inoculated animals}}{\text{N° of animals died within 24-48 hs.}}$

REPORT FROM PROF. S. R. PATTYN
BACTERIOLOGY DEPARTMENT, PRINS LEOPOLD INSTITUTT VOOR TROPISCHE GENEESKUNDE, ANTWERPEN, BELGIUM

Forty batches of mosquitoes have now been inoculated into baby-mice and hamster kidney tissue cultures; these were observed for CPE and haemabsorption. No isolations were made.

Sera of hedgehogs and fowls captured in rural regions around Antwerpen were tested for HI and N antibodies for arboviruses. Results are as follows:

Sera	HI					N	
hedgehogs	WEE	Chik.	SF	YF	RSSE	RSSE	Chik.
4		80		80	80	0	0
5		40		80	160	0	0
6		20		80	160	0	
7		0		20	40	0	0
8		0		0	0	0	0
9		160		160	160	0	0
10		80		320	320	0	0
11		20		40	80	0	0
13					40		0
14					20	0	
15	160	640		160	640		0
16	40	80		40	80	0	0
17	160	320		160	160	0	0
18		20	640	20	160	0	0
19		20	160	20	80	0	0
20		80	320	320	80	0	0
21	320	640		320	640		0
22	80	320		160	160	0	
23	0	20		20	20	0	0
24		160	320	320	80	0	0
25		20	40	160	80	0	0
27		20	80	160	40	0	0
29		0	20	20	20	0	0
6 batches of fowls	0	0		0	0		0

Antigens for the HI test were protamine treated alkaline suspensions of brains from infected newborn mice. Sera were kaolin erythrocytes treated.

Neutralizing antibodies were determined in mouse I.C. tests using 100-300 MLD_{50}, or in plaque inhibition tests (technique of Porterfield) using serum saturated paper discs.

In view of the broad occurrence of antibodies for antigens of the A as well as the B group, we think that chances are that the HI substances in hedgehog sera are non-specific. Sera will be re-tested after acetone treatment.

HA reducing activity of organ extracts of animals on arbovirus antigens, mentioned in info exchange no. 10 was found to be non-specific.

Observations on tissue-virus union in vitro were continued. Several methods of testing are being compared.

A comparative investigation on 10 different group A strains is currently under way. It is the aim to use as many techniques as possible Up until now, a fairly good comparison has been made for plaque production in CETC. Two techniques were used: tris buffered Porterfield technique in petri dishes and $NaHCO_3$ buffered Melnick-Hsiung overlay in stoppered bottles. Two lots of agar were tested: Difco Noble and Oxoid ionagar n°2.

All group A viruses tested (Bebaru, Chikungunya, EEE, Getah, Mayaro, Middleburg, Semliki F., Sindbis, Uruma, WEE) produce plaques under agar. The following main points were made:

° viruses may be classified as producing plaques of 3 sizes:

small (1-2 mm) Bebaru, Getah -
intermediate (2-4 mm) Chik, MB, SF, Si, Ur
large (4-6 mm) EEE, WEE

° no virus strain gave significant better results in bottles in bicarbonate containing medium. Only in the case of MB are the plaques in bottles with very clear cut edges.

° the brand of glass may be of importance. All tests were made on Jena glass petri dishes. In the case of Sindbis we observed definitely greater plaques in anumbra-brand dishes. We hope to investigate this point also for the other viruses.

- for some viruses ionagar is better than noble agar allowing the appearance of clearer plaques, sometimes somewhat earlier or giving somewhat higher counts. This is the case for Bebaru, Sindbis.

- small and intermediate plaques were observed with MB virus (which was obtained as a "large" plaque variant from Dr. Porterfield and had been passed once in babymice and twice in CETC in our laboratory).

Appearance of CPE in CETC tubes was delayed for Getah virus as compared with the other viruses, CPE by Getah virus appearing only after 5 and 6 days.

Three strains have been inoculated by the SC route, into babychicks: MB, Bebaru and Uruma. Uruma virus kills babychicks.

Titrations of blood specimens to detect viremia have not been completed.

The strains were also inoculated into hamsters by the SC and I.C. route with the following results:

	I.C.	S.C.
Bebaru	-	-
Chikunguya	+	-
EEE	+	+
Mayaro	+	+
MB	-	-
SF	+	+
Si	-	-
Uruma	-	-
WEE	+	+

REPORT FROM DR. R. PANTHIER AND CL. HANNOUN
SERVICE DE LA FIEVRE JAUNE ET DES ARBOVIRUS
INSTITUT PASTEUR - PARIS, FRANCE

Ecology of Arboviruses in Southern France

a. Serological surveys on human subjects. Several groups of "normal subjects" (in fact, sera collected from blood donors and not necessarily representing normal population) were tested by HI against 13 strains of Arbovirses (Chik, Sind, YF, WN, DNG 1, DNG 2, NTA, MVE, SLE, Bun, TAH, SFS, SFN).

The first results, grouped by area, are shown in Table I.

Table I

Département	Area	Number of sera	H I + B		H I + TAH		H I + SFS	
			No	%	No	%	No	%
Hérault	Montpellier	394	72	18	62	15	4	1
Gard	Nimes	345	17	5	120	35	8	2
Bouches du Rhône	Arles	184	17	10	49	26	1	0,5
Bouches du Rhöne	Marseille	79	4	5	15	19	0	0

Among the Group B reacting sera, several show a broad group reactivity and some give a monovalent response against WN virus.

b. Serological surveys on animals from Camargue. Several groups of animal sera were also tested. The results obtained with horses are shown in Table II.

Area	Number of sera	H I + W.N.	H I+ TAH.	NT + W.N.
Camargue	47	4	16	12
Controls Lyons and Paris	30	0	0	0

Table II

Some of these horses had been ill in 1962. The presence of antibodies against WN shows that this virus was active in this area, probably a certain time before the sampling since NT is more frequently positive than HI.

c. Virus Isolations. Field studies were organized in June, September, November, and December 1964 to attempt isolation of the viruses, the activity of which had been demonstrated by the serological studies. This was successful for WN virus which was isolated from a pool of *Culex modestus* Ficalbi and from the blood of two entomologists who were bitten by these mosquitoes during the September mission and who presented a few days later a febrile episode.

(Isolement en France du virus West Nile a partir de malades et du vecteur, *Culex modestus* Ficalbi, C. Hannoun, R. Panthier, J. Mouchet et J.P. Eouzan, C.R. Acad. Sci. Seance du 23 Novemvre 1964).

Other Serological Surveys (HI Same Antigens).

a. Morocco (180 sera collected by Dr. Chabaud and Dr. Mailloux). Of 70 sera from Marrakech, El Kelaa, Agadir, and Goulimine, three reacted weakly with WN (10-10-40), one with Sindbis (10). Of 52 sera from Sefrou, four reacted with DNG 2 antigen (80-80-160-320). Of 20 sera from the coastal area, two reacted with WN (10). Of 20 sera from south of Fez, one reacted with DNG 1 (40) and 1 with MVE and SLE (20). Eighteen monkey sera from El Ksiba were completely negative.

The absence of HI antibodies in the majority of these sera seems to indicate that the activity of arboviruses is very small in this area.

b. Spain (Barcelona) (63 sera collected by Dr. Pumarola). Of sera of rice field workers, eight reacted with Tahyna, one with WN.

c. Madagascar (194 sera collected by Dr. Sureau).

1) It was not possible to get evidence of an arbovirus etiology from sera of 53 patients with "dengue-like fever" observed in 1963 in Madagascar. However, 13 of them showed a reaction against group B viruses, especially DNG 1, WN, and NTA; but in absence of adequate paired sera, it was not possible to conclude.

2) One hundred forty-one sera from young adults were also examined and showed a large incidence of group B antibodies.

d. Noumea (New Caledonia) (20 sera collected by Dr. Desmoulins). Three reacted with Chik (10-10-80), 8 with group B antigens (principally MVE and DNG 1), and 3 with TAH (10-20-80). This latter antigen was tested as a representative strain of the California complex.

e. Martinique (136 sera collected by Dr. Mille and Dr. Le Gonidec). Fifty-one isolated sera from patients suffering of the epidemic exanthematic fever were obtained at the time of the dengue epidemic in Jamaica. In more than half the cases, high levels of group B antibodies, principally DNG 1, MVE, and SLE, were found. Eighty-five sera from young normal subjects show a moderate incidence of the same group B antibodies.

f. Guadeloupe (47 sera collected by Dr. Escudie). Group B antibodies are frequent in the convalescent sera of the exanthematic fevers. Normal controls are now studied.

Studies of the Small Plaque Variant of Sindbis Virus.

The results of studies on the small plaque variant of Sindbis virus show the necessity of a longer absorption period for this variant and some "one side" antigenic difference demonstrable by HI using chick antisera.

(Mutants a petites plages du virus Sindbis, C. Hannoun, J. Asso et P. Ardouin, Ann. Inst. Pasteur, 1964, 107, 598-603).

Preparation of Chick Antisera:

The production of antisera for use in HI tests with a good specificity was studied on baby chicks. The optimal conditions were found as follows: One intramuscular injection to one-week-old chicks, another after one week, bleeding the following week. This very simple immunization schedule gave good results for viruses of group A and California complex, and in the group B for WN, CEE, SLE. (Preparation chez le poussin de serums de reference pour la reaction d'IHA avec les Arbovirus, C. Hannoun, L. Chaumont et R. Panthier, en preparation).

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

The serological survey initiated in 1963 in Upper Volta (West Africa, 12°N, 0-4°W) has been completed. The sera were collected in all the stretch of the country, which was divided into five areas: north (toward the border of Mali); east (border of Niger); south (border of Ghana); southwest (border of Ivory Coast); west (border of Mali).

The total number of sera processed by HI test was 1,896 with approximately the same number for each age group between one and 60. Rough percentages of positive tests with sera diluted 1:20 were as follows:

A R E A S	A N T I G E N S						
	CHIK	ONN	YF	UGS	WN	ZIK	BUN
NORD (ZINIARE)	76	76	91	76	61	61	44
EST (DORI)	57	57	74	71	65	39	09
SUD (DIEBOUGOU)	85	85	72	61	58	60	24
S.W. (BANFORA)	79	80	71	52	48	46	05
WEST (BOBO-DIOULASSO)	76	76	76	62	43	64	25

The results show a high frequency of antibodies in group A, B, and Bunyamwera. Even if heterologous reactions induced by multiple infections are numerous in group B, it is yet possible to assume that West Nile virus (or a virus closely related) is prevailing in the east, a dry sub-desertic savanna region, whereas Zika virus (or a virus closely related) is prevailing in the west, a wooded savanna region. High percentages of antibodies for yellow fever must be interpreted in the light of systematic mass vaccination against yellow fever every four years. No cases of the disease have been reported since 1953. In group A, Chikungunya and o'nyong-nyong are uniformly distributed. The titration of antibodies by HI test does not allow to state which of the two viruses is the causative agent. Group Bun is irregularly distributed.

The study of the percentages of positive sera according to age groups shows that from 5 to 10 we find the same figures (more than 70 p 100) as for adults. Quite the contrary, percentages increase gradually according to age for groups B and Bun.

The results of the survey are more detailed in a paper to be issued in December 1964 or January 1965 in "Annales de l'Institut Pasteur."

REPORT FROM DRS. A. CHIPPAUX AND CL. CHIPPAUX-HYPPOLITE

PASTEUR INSTITUTE OF BANGUI
AFRICAN REPUBLIC OF THE CONGO

Attempts at isolation.

A clinical and immunological investigation was based on, during the years 1962 and 1963, sixty observations of exanthematic fevers and various febrile syndromes in two different ecologic zones of the African Republic of the Congo: the northwestern plateau (Bouar) and the region of Bangui, at the northern border of the great equatorial forest.

The immunological study of the curve of HI antibody has shown in 9 cases at Bouar and 8 cases at Bangui the possibility of an infection due to an arbovirus of group A (Chikungunya) or group B (West Nile and Zika). This study is made the subject of an article being published.

After these first results, the investigation was taken up at Bangui in 1964 with the collaboration of the practicing physicians, intensifying the attempts at isolation. From February to August (end of dry season and first half of rainy season), 70 human sera and six human spinal fluids were received and inoculated in parallel on cultures of chicken fibroblasts (Dr. J. Del mon-Orstrom) and into litters of young mice less than three days old.

Up to the present, we have obtained the isolation of three strains of viruses from sera, of which two present certain characteristics of the arboviruses, but for which HI antigen has still not been obtained.

Three strains of virus have been isolated from spinal fluids. One of these has been isolated from a fatal meningoencephalitis. All three are being studied. In one of these cases, as well as in one case in which the virus has not been isolated, there has been noted a significant increase of the Chikungunya and O'nyong-nyong antibodies.

Immunological Investigations.

Five hundred thirty sera of Babinga Pigmies have been collected and studied with the HI test with the gamut of antigens customarily used by the Pasteur Institute of Bangui, obtained from the EAVRI of Entebbe and from the Pasteur Institutes of Dakar and Paris: Chik, Onn., Sem, Sind., F.J. (French neurotropic strain), W.N., Zika, Ug. S., Bun. We have added an antigen prepared by extraction in sucrose-acetone with the strain Bangui M 7 isolated from salivary glands of bats. Almost all of these subjects had eluded the mass vaccination campaigns against yellow fever.

In one of the groups, four sera present exclusively yellow fever antibodies, of which two have high titers. This group of 140 persons comes from encampments closest to their traditional territory and present, paradoxically, the lowest percentages of antibodies.

The other groups present more positive results the closer they come to the edge of the forest, and tend to become sedentary. A publication grouping the results obtained is being prepared.

The various investigations that we presented in our previous report are being continued at the same time with the HI test and by sero-neutralization on mice with the yellow fever virus.

Among the children of Bangui (432 sera) and of Lobaye (447 sera), a relatively large proportion of children, belonging to age groups that have only been touched exceptionally by vaccination against yellow fever present yellow fever antibodies at levels which are often very high, both in the HI and sero-neutralization tests.

A mission has been conducted at the end of the dry season in the eastern end of the country at the Sudan border and the eastern province of the Congo Leo (Obo), and 300 sera have been drawn and sent to the EAVRI of Entebbe.

Study of Potential Virus Reservoirs.

Two regions have been chosen firstly: 1) Bangui and its immediate vicinity; 2) Boukolo-La Maboke in the great equatorial forest 127 km southwest of Bangui (3°7 north latitude, 17°55 east longitude), where there is an experimental station of the Museum of Natural History of Paris.

Inventory of the fauna. We have begun, in close collaboration with the Laboratory of Mammology of the National Museum of Natural History (Dr. F. Petter), the study of the rodents and small tree-dwelling mammals of these two zones. In three years, we have examined about 1,650 rodents (*Rattus*, *Mastomys*, *Lemniscomys*, *Arvicanthis*, *Dasymys*, *Leggadas*, *Lophuromys*, *Thamnomys*, *Praomys*, *Aethomys*, *Hybomys*, *Grammomys*, *Stochomys*, *Ozenomys*, *Cricetomys*, *Euxerus*, *Funisciurus*, *Heliosciurus*, *Anomalure*, *Graphiurus*, *Tatera* *Taterillus*, *Hylomnyscus*, *Atherure*) as well as more than 200 *Crocidura*, 2 *Sylvisorex*, 2 *Suncus*, 6 *Lemuridae*, and 70 *Simians*.

Study of the sensitivity of various species of rodents captured at Boukoko-La Maboke and at Bangui, towards the virus of yellow fever (French neurotropic strain), and towards the West Nile virus (Strain B 956 of the EAVRI of Entebbe. The rodents already tested are: 12 *Lophuromys*, 12 *Praomys*, 3 *Thamnomys*, 10 *Hybomys*, 20 *Mastomys*, 6 *Stochomys*, 5 *Leminscomys*, 4 *Steatomys*, grouped by genera into 16 lots. This work is now completed with the species that seem to us most interesting because of their density or of certain aspects of their ecology.

Attempts at isolation. They are based from 1961 to 1964 on: a) 17 pulverized preparations of mosquitoes and two of ticks; b) the organs of 1,151 small mammals (Muridae and Crocidura) almost exclusively, grouped into 86 pools; c) 16 experiments on sentinel mice and 7 on sentinel monkeys. These animals have been exposed in the fixed biotopes at different heights between ground level and 34 meters (that is to say, less high than the tops of the largest trees of the primary forest).

The blood samples have been tested on young mice and compared to the sera of the sentinels by HI test before and after the experiment. Up to the present, no strain of virus has been isolated.

Isolation attempts have been made on the salivary glands and brains of 64 bats (Mollossidae and Vespertilionidae) grouped in 7 pools. One strain has been isolated from salivary glands of bats captured near the station of La Maboke (named provisionally Bangui M 7). It shows the characteristics of a group B arbovirus and seems, according to the first immunological results, to be in the West Nile subgroup.

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

The good fortune of inheriting a starter colony from the WACMAR Laboratory in Yaba got the University of Ibadan arbovirus program off to a flying start with around 80 newborn mouse groups available per day by the end of July. Spirits soared when isolations of arbovirus agents from human blood began to be made, but were soon dampened when in many passages appeared a mouse colony virus (IB 1141) that killed the inoculated litters in 2 to 4 days and made uncertain the isolation of other agents. Meanwhile, entomological specimens were being inoculated in 5 or 6 day old mice, which apparently when sacrificed for passage are less likely than the younger sucklings to be harboring in the brain the endemic colony virus. In October and November, more than 70 viral agents were obtained from cattle ticks. These appear to represent at least two

antigenic types which are not related either to Group B or to Sogoto virus of East Africa. Representative strains of the tick and human agents have been sent to the RFVL in New York for comparison with world viruses. In Ibadan, the strains are tested with each other and with a limited supply of group sera.

It has been found possible to continue working with the infected mouse colony by treating all fast killing isolates with antiserum to the colony agent. One-injection antiserum, diluted 1 to 4, will completely inhibit 0.02 ml of a 10^{-3} dilution of IB 1141 virus inoculated i.c. The agent is DCA resistant. It is not neutralized by EMC, mouse polio (GD1) or Coxsackie B antisera.

It is planned to substitute a solidly immune colony for the presently endemically infected one, with the hope of preventing reinfection in later progeny. So far, 4000 adults have been immunized and breeding has been started in temporary quarters. Meanwhile, the old colony will go out with litters for inoculation, the males will be destroyed, and the premises disinfected, before moving the new colony into place.

REPORT FROM DR. B.M. MCINTOSH
THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH
JOHANNESBURG, SOUTH AFRICA

Sindbis and West Nile Viruses.

In an attempt to assess the vector capability for Sindbis and West Nile viruses of various common mosquito species occurring on the South African highveld, transmission experiments are being undertaken. After preliminary screening experiments in which it is hoped that non-vectors will be detected, an attempt will be made to work quantitatively although it is evident that success with this approach is very much dependent upon the facility with which the various species will feed in the laboratory.

The intention is to feed mosquitoes on serial 10-fold doses of virus with a maximum of 5.0 logs, mixed with defibrinated blood. The engorged mosquitoes are held for 3 weeks when transmissions are attempted with individual

mosquitoes from the group of mosquitoes fed on the 5.0 log virus concentration. With the mosquito species worked with so far, two-week-old chicks have proved highly suitable indicator-hosts for both viruses. Evidence of transmission has been obtained from a viremia test 48 hours after the mosquito has fed, as well as an HI antibody test 2-3 weeks later. After the transmission feedings are complete, all mosquitoes from each group are tested individually for virus by mouse inoculation. In this manner, a transmission rate (number mosquitoes transmitting/number mosquitoes feeding) is obtained for the highest virus dose, as well as an infectivity rate (number mosquitoes infected/number tested) for all virus doses. By applying the Reed and Muench formula to the ratio of infected to non-infected mosquitoes recorded with each serial virus concentration, an infectivity threshold is calculated which will infect 10% of the mosquitoes. Table 1 shows the results obtained in such experiments with *Culex pipiens quinquefasciatus* (fatigans) with both viruses. For the assessment of vector capability, it is assumed that any infected mosquito will eventually be able to transmit provided that a good correlation exists between the transmission and infectivity rates in the group fed on the 5.0 log virus concentration.

Table 2 shows the results of preliminary screening tests on two other mosquito species. From these tests, it appears that *C. univittatus*, the species from which West Nile and Sindbis viruses have most frequently been isolated, will ultimately be shown to possess a higher vector capability than *C. p. quinquefasciatus*.

The susceptibility of *C. pipiens pipiens* to West Nile virus was somewhat unexpected as, although this mosquito is the most abundant species at our Johannesburg study area, feeds primarily on birds and some 75,000 have been tested for virus, West Nile virus has not yet been isolated from it by us.

In another experiment comparing infection of mosquitoes by inoculation with ingestion, it was not found possible to infect *Aedes aegypti* with 4.9 logs of West Nile virus by ingestion, although by inoculation of the same virus a threshold of infectivity of 3.0 logs was obtained.

Table 1. Quantitative estimation of vector capability of Culex pipiens quinquefasciatus.

Virus	Infecting Dose	Transmission Rate	Infectivity Rate	Infectivity threshold (logs)
SINDBIS	4.7	3/4	18/23	2.8
	3.7	-	8/14	
	2.7	-	2/15	
	1.7	-	0/21	
WEST NILE	5.5	4/5	7/ 7	3.0
	4.5*	-	-	
	3.5	-	2/ 9	
	2.5	-	0/ 6	

* In this group no mosquitoes fed on an infective meal.

Table 2. Viral susceptibility screening tests.

Mosquito	Virus	Infecting Dose	Transmission	Infectivity Rate
C. univittatus	Sindbis	1.7	Pos.	2/10
"	West Nile	3.1	Pos.	4/ 7
"	" "	2.3	Pos.	21/26
C. pipiens	" "	4.9	Pos.	2/ 3
"	" "	3.6	Neg.	2/ 4

GENERAL INFORMATION

Dr. Donald Stamm, Chief of the Virus Ecology Laboratory, Virology Section, died October 21, 1964, after a long illness.

He was born August 12, 1924, in Pottsville, Pennsylvania. He earned his V.M.D. degree in 1948 at the University of Pennsylvania School of Veterinary Medicine and his M.P.H. degree in 1959 at Johns Hopkins University School of Hygiene and Public Health.

After a number of years in private practice in Crane, Missouri, Dr. Stamm was commissioned in the Public Health Service in 1952. He served with the CDC Virus and Rickettsia Section, Montgomery, Alabama, as Assistant Chief of the Veterinary Research Unit until 1959. He became Chief in 1959, and later was appointed Chief of the Virus Ecology Laboratory. At the time of his death, Dr. Stamm was a Veterinary Officer-Director in the Commissioned Corps of the Public Health Service. He was a member of the American Veterinary Medical Association and the Ecological Society of America.

Dr. Stamm's illness forced him to cancel plans for study in New Zealand as a research scholar in virology under the Fullbright-Hayes Act.

EDITORIAL NOTE

The issue of Infoexchange Number Eleven has been delayed by priority occupation of all production facilities with other activity and it is regretted. Deadline for contributions for Infoexchange Number Twelve is July 1, 1965. An additional notice canvassing for contributions will be sent along with a new edition of the participants' list on June 1.

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Editor