

ECOLOGICAL INVESTIGATIONS PROGRAM
ARBOVIRAL DISEASE SECTION
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FORT COLLINS, COLORADO 80521



ARTHROPOD-BORNE VIRUS
INFORMATION EXCHANGE

61

Number Eight

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

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ANNOUNCING A NEW JOURNAL: JOURNAL OF MEDICAL ENTOMOLOGY. This journal will be published quarterly by the Bishop Museum, Honolulu, Hawaii, commencing in early 1964. Each number will contain about 150 pages; thus each annual volume will total about 600 pages. Format will be similar to that of "Pacific Insects". Contents will concern all phases of medical entomology and acarology, including systematics of insects and mites of potential public health significance. Comprehensive articles, up to 100 pages in length, will be given priority. Articles are subject to a page charge of \$10.00 per printed page. This is not to be paid before editorial acceptance of an article, which is determined irrespective of the question of charges. Charges may be paid by Bishop Museum in certain cases, including articles resulting from its program. Two copies of each manuscript, double spaced, are required. Authors will receive 50 reprints, without covers, gratis except for postage. Initially the journal will not be available by exchange. Subscription rates will be \$10.00 per volume to institutions and dealers and \$7.00 to individuals. Price in Japan: ¥3,500 or ¥2,500.

The opinions or views expressed by the contributors do not constitute endorsement or approval by the U.S. Government, Department of Health, Education, and Welfare, Public Health Service, Communicable Disease Center.

EDITORIAL NOTES

Herewith is the eighth issue of the Arbovirus Information Exchange which required two months to prepare for printing. Not only has increased size required more time for transcription onto stencils, but many of the tables were received as carbon copies or in such condition as to require complete retyping. The number of contributions requiring translation into English also increased. Compared to the first issue of forty pages prepared three and a half years ago, which contained twenty contributions, only three tables, and a list of fifty-two participants from nine countries, issue number eight contains more than 130 pages, 51 tables, and accompanies a separate list of 171 participants in 46 countries, some of which are contributing for the first time.

The more technical nature--as represented by the large number of tables--does not only reflect the increasing sophistication of arbovirology as a science, but the greatly expanded activity of scientists and virus laboratories all over the world. In accordance with the original direction of the arbovirus investigators who initiated the Exchange, every effort has been made to keep the Infoexchange timely, comprehensive, and informal. Efforts will continue to keep it as a quick and convenient mechanism to maintain global communication among an international fraternity of scientists. Henceforth, this will require that any tables submitted for inclusion be in the original and clearly typed so that they can be photographically reproduced without time consuming retyping.

We hope that you all share the pride of accomplishment as the unusual concept of an Arbovirus Information Exchange continues to grow in substance, influence, and usefulness. Requests for contributions to issue nine will be mailed in December with deadline for contributions being January 31, 1964.

Telford H. Work
Editor

REPORT FROM THE SUBCOMMITTEE ON THE RELATION OF BIRDS
TO ARBOVIRUSES, OF THE
AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

A meeting was held in conjunction with the annual meeting of the American Ornithologists Union in Gainesville, Florida, August 14, 1963. The major part of the day was spent in rewriting a review paper on bird-arbovirus relationships. This paper will be submitted to an ornithological journal within a few weeks.

Dr. Davis is preparing a manual for field studies on bird-arbovirus relationships. A first version has been circulated through the subcommittee and revised according to their suggestions. The manual is being used in Dr. Davis' field program this summer; it will be further revised according to experience gained in its use there. The manual will then be made available to other persons doing similar work and their suggestions included in the final published version.

Dr. Stamm and Dr. Newman have collaborated on a paper, "Evidence of Southward Transport of Arboviruses from the U. S. by Migratory Birds", to be presented at the Seventh International Congresses of Tropical Medicine and Malaria in September 1963 at Rio de Janeiro.

Dr. Hickey reports that the Bird-banding Office of the U.S. Fish and Wildlife Service at Patuxent Wildlife Research Center, Laurel, Maryland, is assembling information on aging and sexing species of North American birds.

Dr. Hickey and Dr. Stamm will be out of the U. S. in 1964 and it was therefore necessary to make plans to adjust the future membership of the subcommittee. It was decided to enlarge the subcommittee to six members and to replace two members each year. Those mentioned above will be the first two to be replaced. Three new persons are being invited to join the subcommittee and their names will be announced following our next meeting in Chicago, November 6-9, 1963. Ex-members will receive copies of inter-subcommittee communications and are invited to make suggestions to the active group.

D. D. Stamm, Chairman
D. E. Davis

J. J. Hickey
R. J. Newman

M. W. Provost

REPORT OF THE CHAIRMAN OF THE SUBCOMMITTEE ON
ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Arthropod-borne Virus Catalogue:

A total of 87 catalogues have now been issued; 35 to institutions and laboratories within the continental United States, one in Canada, and 51 overseas, representing a total of 31 countries.

During the first half of this year, 11 cards of previously unregistered viruses were issued, bringing the total of viruses now in the catalogue to 139. It should be emphasized that the registration of viruses is voluntary and is dependent upon the interest and participation of the person, or persons, making the original isolation. There are perhaps a dozen viruses which have been recently discovered and referred to in annual reports, that have not been registered. We have not attempted to secure registration of the different sero-types of a clinical entity that have been described in such animal diseases as Blue Tongue and African horse sickness. If these were to be included as distinct species, the number of known arboviruses would be increased by an additional twenty or more.

The number of abstracts that have been issued on 3 x 5 slips now totals 2,067. A total of 1,197 of these were from Biological Abstracts (BA) covering the period from 1957 to July 1963, and 870 from the Bulletin of Hygiene (BH) and Tropical Diseases Bulletin (TDB) for the period 1959 to July 1963. Four hundred thirty-five of the Abstracts are duplicates; that is, abstracts from BA and from BH or PDB. The reason for this duplication is that in the latter two abstract journals, the abstracts represent opinions of the reviewing staff, while the BA abstracts are, with few exceptions, by the author(s) of the article abstracted. In addition, 71 current information slips representing personal communications, or unpublished information have been distributed.

REPORT FROM DR. M. MARTINS DA SILVA
PAN AMERICAN HEALTH ORGANIZATION, WASHINGTON, D. C.

Concurrent with the VII International Congresses of Tropical Medicine and Malaria, the Pan American Health Organization, through

its Office of Research Coordination, and with the collaboration of Dr. William F. Scherer, Cornell University Medical College, and Dr. William C. Reeves, School of Public Health, University of California, convened in Rio de Janeiro, Brazil, last September, two one-day meetings on: a) Arbovirus Problems in the Large River Basins of Equatorial South America (5 September), and b) Recent Arbovirus Epidemics in the Americas and Information Exchange Activities (7 September).

These meetings had five primary objectives:

- 1) To bring together arbovirus research workers interested in the large river basin areas of equatorial South America, and to give them an opportunity to exchange information on each other's research activities.
- 2) To assemble knowledge on arboviruses in these river basins, particularly as they relate to current and potential health hazards to man and animals.
- 3) To provide orientation on the purposes and aims of current world-wide procedures for information exchange on the arboviruses and to review and discuss the present status of services and functions of regional and world reference laboratories for the arboviruses.
- 4) To review arbovirus epidemics that have developed in the Americas in 1962 and 1963, with particular attention to their magnitude, the application of available resources to their investigation, recognition, of the limiting elements and an assessment of the data obtained.
- 5) To explore possible ways in which PAHO may assist laboratories or countries of the Western Hemisphere in either their own or collaborative arbovirus research and research-training efforts.

The meetings were held at the Institute of Microbiology of the University of Brazil. There were 111 participants to the sessions representing nine countries of the Americas and Belgium, Czechoslovakia, England, India, Japan, New Zealand, Senegal, Thailand, USSR, and international organizations and foundations.

The report of the meetings is being distributed to the participants. Other scientists interested in and concerned with these fields of research may request copies from:

Office of Research Coordination
Pan American Health Organization
Washington, D. C. 20036

During the second meeting of the Advisory Committee on Medical Research of PAHO, which took place in Washington, D. C., 17-21 June, the choice of the Instituto Adolfo Lutz, Sao Paulo, as a regional reference center for arboviruses was approved.

REPORT FROM DR. WIEBENGA
FOR THE LABORATORY OF TROPICAL VIROLOGY
AND MIDDLE AMERICA RESEARCH UNIT

EPIDEMIC HEMORRHAGIC FEVER, BOLIVIA, 1963

The previous issue of the Infoexchange reported observations of epidemic hemorrhagic fever in Bolivia during 1962 and laboratory evidence that the disease in Bolivia was probably caused by a virus related to Junin virus, isolated from cases of "Epidemic Argentinian Hemorrhagic Fever".

A major collaborative study was conducted this year by the staff of Middle America Research Unit (MARU) in Bolivia and the Laboratory of Tropical Virology (LTV) staff in Bethesda. A field team composed of Dr. Johnson, Drs. Mackenzie, Kunz, Yunkers, etc., worked in Bolivia during May, June, and early July. Drs. Johnson and Mackenzie and Mr. A. Munoz were apparently exposed and infected during their studies. They were air-evacuated to Gorgas Hospital, C.Z., with clinical disease typical of "Bolivian hemorrhagic fever". Their complete recovery has been observed with relief.

Among others still to be identified, a "virus" was isolated from the spleen of a fatal case. Isolation and passage of this agent was accomplished by inoculation into suckling hamsters (SH) by a combined intracerebral and intraperitoneal route. Hamster spleen seemed to be the preferable source of virus for serial passage. Specimens of this agent were brought to Bethesda by Dr. Johnson. Additional passages to SH/4 were used for preparation of a CF antigen. Hamster brains harvested 8 days after inoculation, sucrose-acetone extracted, and treated with 0.4% beta-propiolactone by Major R. McKinney, USAMRU, Ft. Detrick, proved most suitable for our immediate purpose.

Fig. 1 summarizes the result of 3-way block titrations with antigens and "antisera" produced with the Bolivian isolate (BHF) and Junin and Tacaribe viruses. BHF virus and antiserum were obtained from hamsters; reagents for the other 2 viruses were obtained from infected SMB and immune mouse ascitic fluid (IAF). The 3 viruses seemed indistinguishable by this test.

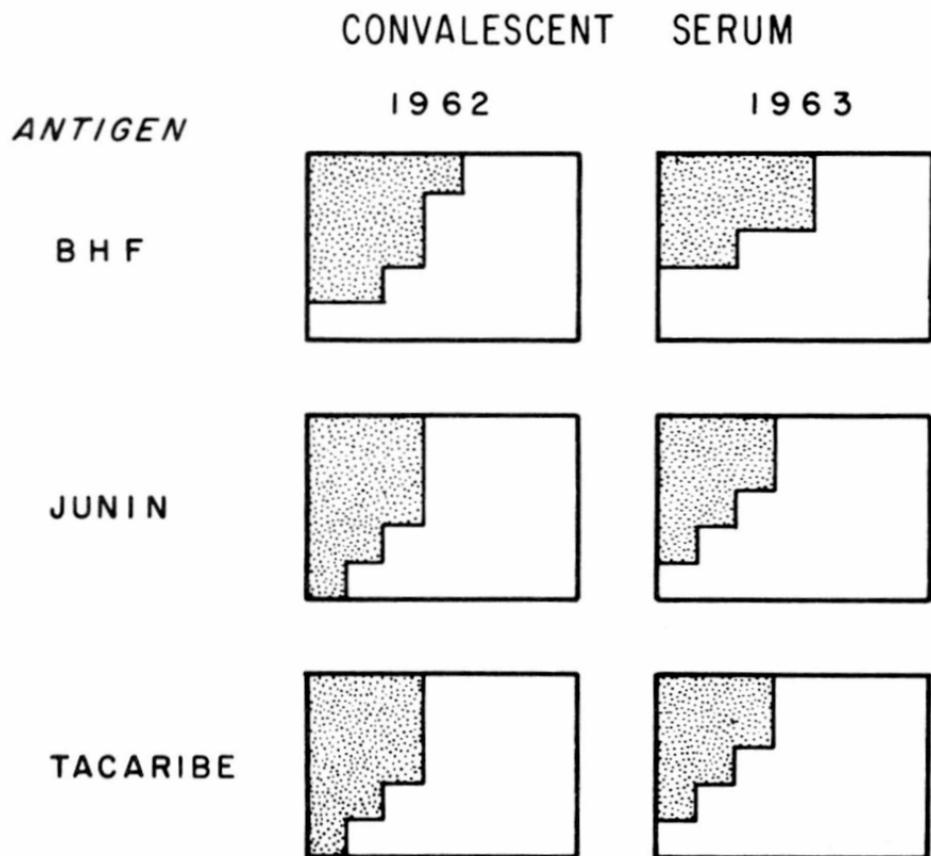
A similar test with these antigens was done with two convalescent sera selected from the 1962 and 1963 collections, respectively. Results of this test, shown in figure 2, also indicate serological crossing.

Early convalescent sera (1 month) obtained from Drs. Johnson and Mackenzie and Mr. Munoz demonstrated serological conversion when tested with the BHF antigen. Prebleedings obtained about 1 week prior to onset of illness were less than 1:4.

Serological conversions have also been demonstrated in serial serum specimens obtained from clinical cases by the MARU team in Bolivia this year. Of 57 cases, sixteen have shown definite rise in CF serum titer, but laboratory testing of all sera is incomplete at the time of this writing.

CF antibody has also been demonstrated in some rodent sera; further work with rodent materials is underway in the laboratory and in the field.

Results obtained thus far support last year's clinical, epidemiological, and laboratory evidence that directed attention to a virus related to Junin virus, previously reported from Argentina as a cause of a new epidemic hemorrhagic fever.



ANTIBODY

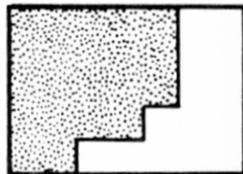
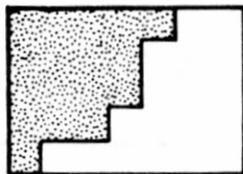
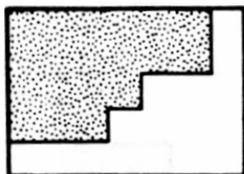
ANTIGEN

BHF

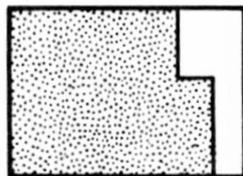
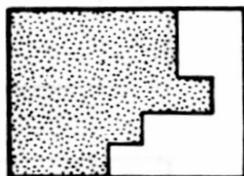
JUNIN

TACARIBE

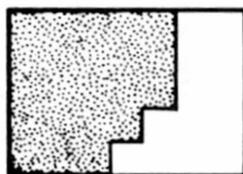
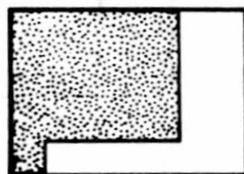
BHF



JUNIN



TACARIBE



REPORT FROM DR. G. H. BERGOLD
HEAD, VIROLOGY DEPARTMENT
INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS
CARACAS, VENEZUELA

Yellow fever has been found (Sellers) to produce much less CPE in baby hamster kidney cells (BHK 21) than VEE, Mayaro, Ilheus, and SLE; on the other hand, cultures infected with yellow fever produced interferon active against virulent and attenuated FMD virus.

BHK 21 cells have proved to be very suitable for primary isolation and typing of VEE virus and screening of neutralizing antibodies. However, owing to the high sensitivity of the cells to arboviruses, neutralizing titers are low compared to KB cells and suckling mice tests (i. p.).

Previously it was reported that BHK cells are not suitable for viruses forming plaques after 5 days; however, recent experiments (Bergold) using an enriched overlay (Miles and Austen, Australian Journal of Science, 25: 456, 1963) in connection with lower concentrations of agar (0.5%) and bovalbumin V fraction (0.14% dry weight/total overlay) resulted in the formation of round and well recognizable plaques of yellow fever (French Neurotropic). These were made visible by staining on the fifth day with neutral red in a second overlay of the same composition. Presently all available yellow fever strains are being tested for the production of plaques. For these experiments Falcon Plastics disposable tissue culture flasks (30 ml with screw tops) have been used.

In connection with new outbreaks of VEE in Venezuela, further investigations (Sellers, Bergold, Suarez, and Morales) were carried out on mosquitoes and blood samples collected in January 1963 (Information Exchange No. 7) in Sinamaica. No virus was isolated but HI and neutralizing antibodies were found in patients previously affected, in Didelphis marsupialis, a goat, and possibly a rat. In addition, antibodies were observed by HI in a pigeon.

No virus could be isolated in a collection of mosquitoes from Sinamaica and Villa del Rosario (May, 1963). However, VEE was isolated from 6 sera and 3 throat swabs of patients from Villa del

Rosario, Monay and Maracaibo, as well as from a brain of a donkey from Maracaibo. The isolation of VEE from throat swabs confirms results previously reported by H. Fossaert from the Instituto de Higiene in Caracas. HI antibodies were found in another goat from the Rosario area. Details of the above are summarized in Tables 1 through 3.

Since January, 1963, the Entomology Laboratory (Suarez) has been engaged in creating conditions for the laboratory adaptation of Aedes taeniorhynchus, since this species was found plentiful in Sinamaica acting as transmitter of VEE. This species is known to develop in saline marshes, but, however, 10,000 eggs were collected in Sinamaica, which is far away from the coast. Consequently, it has been tried to find an essentially salt-free medium suitable for laboratory rearing of this species.

Known previous experiences about rearing of A. taeniorhynchus are based on the exploitation of the feeding conditions of its normal habitat. In July, 1963, it was possible for the first time to obtain a development of this species with a first generation, through the following procedure: the 10,000 eggs collected in the field were activated with NaCl (0.15%) + sucrose (1%) solution in water bath. Eggs were laid on moist Sphagnum moss layered on a cup, and first instar larvae collected on the 15th day by flooding the moss. After hatching, larvae were reared in one lt. cups containing a solution of powdered dog biscuit (0.1 gm) in distilled water. Larvae pupated after a further 7 days, and the overall efficiency from eggs to adults so far has been of 75%. Male adults are fed with pure honey and females allowed to suck on healthy, adult albino mice.

MOSQUITO COLLECTIONS (TOTAL: 5179)

C=captured; T=tested; I=isolation (+ positive, - negative); *=provisional classification

Species	Nov. 1962			Jan. 1963			May 1963			June 1963					
	C	T	I	C	T	I	C	T	I	Rosario			Monay/Maracaibo		
										C	T	I	C	T	I
<i>Aedes scapularis</i>	0			3			4	4	-	71	45	-	9	9	-
<i>Aedes serratus</i>	51	13	+	0	4	-	0	0		6	6	-			
<i>Aedes taeniorhynchus</i>	2032	1599	+	373	260	-	870	870	-	46	44	-	32	32	-
<i>Aedomyia squanipenna</i>	0									2	2	-			
<i>Anopheles aquasalis</i>	41	40	+	483	417	-	3	3	-						
<i>Culex pipiens fatigans</i>	0									369	231	-			
<i>Culex duplicator*</i>	0									602	402	-	4	4	-
<i>Mansonia titillana</i>	0			13						6	3	-			
<i>Psorophora ferox</i>	0									8	8	-	1	1	-
<i>Psorophora confinnis</i>	132	40	+	5	1	-				7	4	-	3	3	-
<i>Psorophora cilipes</i>	3														
Totals	2259	1692		877	682		877	877		1117	745		49	49	

VEE Investigations in Venezuela 1962-63

P=positive; N=neutralization; 0=negative; HI=hemagglutination inhibition

Date of Collection	Locality	Humans			Animals			Mosquitoes	
		Serum	Phar. Swab	Brain	Donkey Horse Brain	Birds	Others	No.	Lots
Nov 8-21	Sinamaica (S)	25/21I	-	2/2P	1/1P	22/0	-	2259	50/11P
Jan 18-31	Sinamaica (S)	10/10N*	-	-	-	12/0	47/3N**	615	20/0
		10/10HI					47/3HI		
May 1-3	Sinamaica (S)	-	-	-	-	-	-	872	9/0
June 13-17	Rosario (R)	-	-	-	-	2/0	13/0/1HI	932	42/0
	Rosario (R)	4/1P	4/2P	-	-	-	-		
June 22-29	Monay (T)	3/3P	3/2P	-	2/0	-	-	49	4/0
	Maracaibo (M)	3/1P	3/0	-	1/1P	-	-		

*10HI = 2x5120, 7x2560, 1x1280; **3N = rat?, Didelphis marsupialis, goat; 3HI = pigeon (1280),

Didelphis marsupialis (2560), goat (160), 1HI = goat (1280)

ANIMALS CAPTURED IN JULY

REPTILIA		MAMMALIA	
Iguanidae		Molossidae	
<u>Iguana iguana</u>	13	<u>Molossus major major</u>	21
Ameibidae		Didelphidae	
<u>Tupinambis nigropunc-</u>	1	<u>Didelphis marsupialis</u>	1
<u>tatus</u>		Muridae	
<u>Cnemidophorus lemnis-</u>	13	<u>Rattus norvegicus</u>	4
<u>catus</u>		Canidae	
Colubridae		<u>Canis familiaris</u>	1
"culebra sabanera"	2	Felidae	
(non-poisonous)		<u>Felis catus</u>	2
AVES		Equidae	
Ardeidae		<u>Equus asinus</u>	3
<u>Leucophoryx thula thula</u>	13	Bovidae	
<u>Florida caerulea caeru-</u>	1	<u>Capra sp.</u>	3
<u>lea</u>		<u>Bos taurus</u>	3
Phasianidae		Suidae	
<u>Gallus gallus</u>	1	<u>Sus scrofa</u>	1
Recurvirostridae			
<u>Himantopus himantopus</u>	4		
<u>mexicanus</u>			
Columbidae			
<u>Columba sp.</u>	12		
Icteridae			
<u>Cassidix mexicanus</u>	1		
<u>peruvianus</u>			
Cathartidae			
<u>Coragyps atratus</u>	1		

REPORT FROM DR. ENRIQUE PRIAS LANDINEZ,
 IN CHARGE OF THE ARBOVIRUS LABORATORY
 INSTITUTO NACIONAL DE SALUD, BOGOTA, COLOMBIA

In order to investigate the possible role as hosts played by birds in the epidemic of Venezuelan equine encephalitis (VEE) which occurred in La Guajira last year, 358 birds were captured and bled from July 4, 1963 through July 18, 1963. Birds were caught in Japanese "mist" nets, bled from the heart or the jugular vein into 2 cc syringes moistened with a 1:200 heparin solution. The plasma was diluted 1:2 to 1:6 whenever the amount obtained was insufficient to submit them to the HI test against acetone-ether extracted VEE and neurotropic French (NF) yellow fever antigens.

Forty-two species belonging to 24 families of birds were studied; only one bird out of 11 of the same species (Saltator coerulescens plumbeus) was positive at the 1:10 dilution against the VEE antigen used.

In contrast, out of the 358 sera tested against the NF strain of yellow fever antigen, 36 were positive, of which one was positive at the 1:10 dilution, 3 at the 1:20 dilution, 16 at the 1:40 and 16 at the 1:60 or plus dilution.

Unless the reactions obtained are nonspecific, it seems that in the near past there has been some B group virus activity in the area. If that is true, then there is reason to believe that the absence of reactors against VEE only 8 months later than the epidemic is a sign that many of the birds were not implicated in it either because they are not susceptible or because those that were infected succumbed.

The places where birds were caught are evenly distributed through the area of La Guajira where human cases of VEE occurred last year and were selected to represent the various ecologic situations. So there was inclusion of the coastal marshy area, the midland xerophytic forest near rivers, ponds or marshes, and the Savannah sections. It was in places richer in vegetation where positive reactors were found: 5 out of 113 in Mayapo, 11 out of 48 at Cavallause (Anjero), 19 out of 47 at Jaguey Caracas and one out of 45 at Cousirijuna. Two other places, Maguasira and Sipa (Jamichera) gave no reactors out of 13 and 92 birds respectively.

Two Columbidae species (Scardafella squamata ridgwayi and Columbigalina passerina albivitta) with 25.3% and 5.4% of reactors were the most abundant species and more evenly distributed through La Guajira. In contrast, Tiaris bicolor omissa and Coryphospingus pileatus brevicaudus species, also abundant, were negative. Specimens of Leptotila verreauxi verreauxi, Aratinga acuticaudata haemorrhous, Furnarius leucopus longirostris, Sakesphorus canadensis phanolencus, Sublegatus arenarum atrirrostris, Polioptila plumbea plumbiceps and Coereba flaveola luteola, showed reactors to yellow fever antigen. Each of the 358 sera were inoculated intracranially into baby mice and no virus was isolated.

REPORT FROM DR. CARLOS SANMARTIN, UNIVERSIDAD DEL VALLE
FACULTAD DE MEDICINA, CALI, COLOMBIA

Three visits were made to Guajira peninsula in connection with the VEE virus epidemic observed in that area from October to December, 1962. On the second trip, second week of December, 1962, Kairi virus was isolated from local mosquitoes. Details concerning this new

isolation of Kairi have been already sent to Dr. Richard M. Taylor for distribution to the recipients of the catalogue.

In the last visit, first week of April, 1963, 163 persons were bled and their sera tested for VEE virus by hemagglutination inhibition test. In the adjoining table the obtained results are presented. The same sera, when tested for Kairi virus antibodies, showed immunity in the local population in the neutralization test but none by the complement fixation.

Hemagglutination-inhibition tests with VEE virus antigen and human sera collected in Guajira during 1st week of April, 1963

Age (years)	Sick*			Not Sick			Grand Total		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
1-4	1/1	3/4	4/5				1/1	3/4	4/5
5-9	10/16	12/19	22/35	1/4	0/1	1/5	11/20	12/20	23/40
10-14	10/18	4/8	14/26	2/5	1/6	3/11	12/23	5/14	17/37
15-19	7/10	2/3	9/13	3/4	1/2	4/6	10/14	3/5	13/19
20-29	8/9	5/5	13/14	2/2	2/4	4/6	10/11	7/9	17/20
30-39	5/5	10/12	15/17	2/3	3/3	5/6	7/8	13/15	20/23
40-49	1/1	3/3	4/4	3/3	0/1	3/4	4/4	3/4	7/8
50 or more	2/2	3/3	5/5	2/4	1/2	3/6	4/6	4/5	8/11
Total	44/62	42/57	86/119	15/25	8/19	23/44	59/87	50/76	109/163
%	70.97	73.68	72.27	60.00	42.11	52.27	67.82	65.79	66.87

*Sick and Not Sick make reference to febrile illness experienced or not during the VEE outbreak of October-December 1962.

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

REPORT FROM DRS. L. SPENCE, T. H. G. AITKEN, C. B. WORTH,
A. H. JONKERS, AND E. S. TIKASINGH, OF THE
TRINIDAD REGIONAL VIRUS LABORATORY

The first half of 1963 witnessed an unprecedented number of virus isolations in Bush Bush Forest, our main site of field operations. By the end of June, 150 viruses had been isolated as compared to 130 during all of 1962. This can probably be accounted for by the following factors:

1) An abnormally wet "dry" season which no doubt maintained breeding places for mosquitoes which otherwise would have disappeared; 2) A greater concentration of effort in a particular section of swamp; and 3) The reduction of pool size for Culex sp. #9 to a maximum of 25 mosquitoes. Table 1 presents the number of isolates from mosquitoes for the first six months of 1962 and 1963 together with the total number inoculated.

Culex sp. #9 continues to be the most frequent source of virus. As such, it is a very good sentinel indicator of virus activity, better than the sentinel mice. Of a total of 150 isolations (Jan. - June 1963) from Bush Bush Forest, this Culex mosquito yielded 114 strains of virus (Table 2). A study of the bionomics of this important species is in progress.

In the last Arthropod-borne Virus Information Exchange (#7, March 1963), we reported the isolation of a virus (TRVL 42336) from Ornithodoros capensis ticks collected on Soldado Rock, a bird-island off southwest Trinidad. O. capensis is a bird feeding tick and since it harbored a virus, the tick might be infecting the bird population of the island. In an effort to study the immunity status of the birds, which are mainly terns (Sterna fuscata and Anous stolidus), three expeditions to the rock were made in January, April, and June. A total of 299 birds were bled. Neutralization tests are incomplete, but suffice it to say that a number of both species of terns were found to be positive to TRVL 42336. This virus is closely related to an agent isolated from Dry Tortugas Island by the Rocky Mountain Laboratory workers.

Studies of virus circulation in laboratory-reared wild rodents are in progress. Oryzomys and Zygodontomys rats will circulate Guama virus (TRVL 33579) on days 2, 3, and 4 p.i. with titers between 10^{-3} and 10^{-5} . Similar results were obtained with Caraparu virus (TRVL 34053-1).

A project started towards the end of last year on the preparation of hyperimmune sera and ascitic fluids in mice (using adjuvant and Sarcoma 180 cells) to our prototype viruses is nearing completion. Results to date indicate that large quantities of fluids can be produced (at an average of 5 ml. per mouse) with relatively high titers (up to 1:1024 in some instances in CF) by this method. We have also prepared a number of "group" hyperimmune ascitic fluids. CF results have shown that these fluids are group specific.

TABLE I

Mosquitoes inoculated Jan. June 1962 & 1963 by month of collection

Month	<u>1962</u>			<u>1963</u>			<u>1963</u>			<u>1963</u>		
	<u>Culex #9</u>	<u>Other Mosquitoes</u>	<u>Isola-tions</u>									
January	61	8	0	6,946	89	1	687	23	5	13,715	168	1
February	166	6	1	7,047	109	0	445	25	5	6,247	84	0
March	293	6	4	5,573	81	1	985	31	3	5,525	78	0
April	134	21	1	1,838	72	0	2,580	65	9	5,476	88	7
May	926	59	12	11,752	191	1	4,904	214	42	9,838	168	2
June	361	28	11	7,889	170	0	2,607	215	50	9,558	208	3
TOTAL	1,941	128	29	41,045	712	3	12,208	573	114	50,359	794	13

TABLE 2Arbovirus Isolations (TRVL) January - June 1963

Virus	S O U R C E					Total
	Culex sp. #9	Other mosquitoes	Rodents	Sentinel mice	Ticks	
VEE	49	5	2	8		64
Una		1				1
Group C	6		2	1		9
Guama gp.	54	3	1	7		65
42520-1-5 type			1			1
42336-type					2	2
Unidentified	5	4		1	1	11
	114	13	6	17	3*	153

* From Soldado Rock; all other viruses isolated from Bush Bush Forest.

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

The search for arboviruses and the epidemiological investigation of infections caused by these, entail one of the chief objectives of the Department of Microbiology, University of the West Indies. The project is financed jointly by The Rockefeller Foundation and the University of the West Indies.

Of paramount importance in the past year was an outbreak of eastern equine encephalitis (EEE) in St. Thomas, one of the eastern parishes of the island. In this outbreak, there were 70 deaths of equines and 9 human deaths out of 11 cases. Seven strains of the EEE virus were isolated, all from brain specimens--3 equine and 4 human. The search for the vector (or vectors) of this virus is still one of major interest. Rodents, mosquitoes, and birds are being investigated. However, during the search, two Cache Valley-like viruses were isolated, one by us from Anopheles grabhami and the other by Trinidad Regional Virus Laboratory (TRVL) from a pool of Aedes taeniorhynchus.

The highlight of 1963 regarding arbovirus investigation is that of an epidemic of dengue. Approximately fifty-six clinically diagnosed and laboratory confirmed cases have been investigated (laboratory confirmation by the hemagglutination inhibition test). The isolation of this virus is at present our main objective. Suckling mice, tissue cultures: HeLa cells, human embryonic lung fibroblast (HEF) thyroid cells and duck embryonic fibroblasts are the hosts which have been used for the isolation of this virus, but with no success.

Since this virus laboratory is the only one in the island, work is not limited to the arboviruses; all epidemics of viral etiology are investigated. This has resulted in the isolation of four type A influenza viruses and several confirmed immunological conversion to type A influenza virus.

Our attention is now focussed on the establishment of the EEE vector and the isolation of the dengue virus.

REPORT OF THE PAN AMERICAN HEALTH ORGANIZATION ON AEDES AEGYPTI ERADICATION IN THE AMERICAS

(Ed. Note: In view of the recent activity of dengue in Jamaica and Puerto Rico, it was suggested by Dr. Earl Chamberlayne of the Pan American Health Organization that timely information regarding the present status of Aedes aegypti might be presented by quoting the pertinent material included in the recently published 1962 Annual Report of the PAHO Director. With Dr. Chamberlayne's permission, this information is presented verbatim from the report.)

YELLOW FEVER CONTROL AND AEDES AEGYPTI ERADICATION

Yellow Fever

During 1962, 52 cases of jungle yellow fever were notified in the Americas--in Brazil, Colombia, Peru, and Venezuela (Figure 2). There were no unusual epidemiological manifestations during the year.

The Organization continued to assist the Yellow Fever Section (formerly Carlos Finlay Institute for Special Studies) of the National Health Institute in Bogota, Colombia, which produces 17D yellow fever vaccine and conducts epidemiological studies on yellow fever and other arbovirus infections. The Organization also continued to assist the Oswaldo Cruz Institute in Rio de Janeiro, Brazil, in producing 17D vaccine and in providing free diagnostic services to other countries in the Americas. Total 1962 production of yellow fever vaccine in Colombia was 716,468 doses, and in Brazil 4,958,000 doses; details regarding use are shown in Table 9.

Aedes aegypti Eradication

The XVI Pan American Sanitary Conference urged the countries and territories still infested with Aedes aegypti to give maximum priority to the provision of funds to complete their eradication campaigns, and requested the Governments of areas where the vector has been eradicated to maintain active surveillance programs to prevent reinfestation.

In the Caribbean, for geographic, economic, and administrative reasons, it has not been possible up to now to coordinate the campaigns

in the various territories and countries so as to prevent repeated reinfestations. In addition, A. aegypti strains resistant to chlorinated insecticides are rapidly developing in many of the countries and territories.

To meet this situation, it was considered essential to make a general review of the campaign in the area, in order to correct existing faults, intensify the pace of activities, and coordinate the various programs in such a way as to make it possible to eradicate the mosquito more or less simultaneously throughout the area in the shortest time possible.

As a first step in this plan, in 1962 the Organization sent an entomologist to study the problem of vector resistance to insecticides in the Caribbean. With A. aegypti larvae from Jamaica and elsewhere in the Caribbean, the entomologist is carrying on susceptibility tests with various insecticides and studying new eradication techniques suitable for the Caribbean area. However, to carry out the eradication plan it will also be necessary to examine the other technical, administrative, economic and political difficulties that are hampering the progress of the campaign in this area.

The Governing Bodies of the Organization have declared A. aegypti eradicated from Bolivia, Brazil, British Honduras, Chile, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Honduras, Nicaragua, Panama, the Panama Canal Zone, Paraguay, Peru, and Uruguay. No new country was added to this group in 1962.

A summary of the status of the campaign in the countries and territories of the Hemisphere is given below, as well as in Table 10 and Figure 3.

Argentina. Eradication activities in this country covered the entire presumably infested area, which measures some 1,000,000 square kilometers. During the initial survey 3,741 localities were inspected, of which 165 were found infested with A. aegypti. All of these localities were negative in the latest verifications made. At the end of 1962, to declare the entire country free of the mosquito, only the investigation of one island in the La Plata River estuary and a special verification of 77 of the 165 originally positive localities still remained to be done.

Colombia. In 1961 Colombia was considered to have eradicated A. aegypti, but in September of that year the city of Cucuta, near the Venezuelan border, was found reinfested. Reinfestation was high and widespread when the first foci was found, but the Government immediately resumed eradication activities with PASB assistance, and the verification completed in December 1962, covering 22,274 of the 22,454 houses in Cucuta, revealed only 14 houses infested with A. aegypti. Four houses in the small community of San Luis, one kilometer from Cucuta, were also found reinfested in 1962. The locality was rapidly treated.

Cuba. Despite administrative difficulties which delayed activities during the first months of 1962, the campaign continued to progress satisfactorily. By December the campaign was in full development in the Provinces of La Habana, Pinar del Rio, and Matanzas, to be extended at a later date to the remainder of the country. Since December 1961, when dieldrin was selected for spraying in Greater Havana and neighboring municipalities because of the low susceptibility of A. aegypti to DDT, the mosquito has been progressively eliminated in the entire working area. As of December 1962, a total of 677 localities had been surveyed in Cuba and 496 found infested. Of the initially positive localities, 409 had been verified after treatment; 155 continued positive.

Dominican Republic. Susceptibility tests made in 1962 with A. aegypti larvae gathered from various localities showed that resistance of the mosquito to chlorinated insecticides was present throughout the entire country. It was therefore decided to interrupt the campaign in November 1962, until such time as the studies underway in Jamaica indicate which insecticides should be used instead of the chlorinated.

Haiti. The campaign was suspended for financial reasons in 1958; infestation at present is probably high and extensive. The emergency public health plan that the Government of Haiti presented to the Tripartite Committee of the Alliance for Progress in 1962 included A. aegypti eradication, and it was hoped that the campaign might be resumed in a not too distant future.

Jamaica. Because of unsatisfactory results, the Government halted the campaign in 1961, to resume it after suitable reorganization. Investigations made in 1962 showed the island to be extensively infested and that in some areas A. aegypti was resistant to the chlorinated insecticides. Studies made during the last quarter of 1962 by an

entomologist sent by the Organization showed that this resistance was higher and more widespread in some areas than had been originally believed. These studies, however, have not clarified the problem fully nor indicated which insecticides could be successfully substituted for the chlorinated hydrocarbons.

Mexico. The campaign has covered all the presumably infested area, where 4,235 localities were surveyed and 600 were found positive. According to the latest inspection, the 600 originally positive localities were considered free of the mosquito. The special verification of the country was started with assistance from the Organization in October 1961, and as of December 1962 a total of 178 localities had been inspected; of these, only the city of Merida was found positive, with infestation of 20 of the nearly 47,000 houses. The city was treated again and the reinfestation eliminated.

Trinidad and Tobago. These two islands had been considered negative for some time, but in 1961 A. aegypti was found in one of the localities of the interior as well as around the harbor of Port of Spain. The necessary measures to eliminate the mosquito from the two areas were taken and Trinidad once again became negative. In 1962, A. aegypti foci were repeatedly found in and around the dock area of Port of Spain, which led to the supposition that the mosquito was being reintroduced by boats arriving from other Caribbean ports. In August the source responsible for the reinfestation was found to be some small vacation islands, belonging to Trinidad, which lie close to Port of Spain and which had never been surveyed or treated. All of these islands were sprayed, and it was expected that the mosquito would soon be eradicated. Tobago continued to be considered free of the vector.

United States of America. According to the latest data available, the areas infested with A. aegypti include the States of Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, South Carolina, Tennessee, part of Texas, the Commonwealth of Puerto Rico, and the U.S. Virgin Islands. The XVI Pan American Sanitary Conference was informed that the Government intended shortly to initiate A. aegypti eradication activities in the continental United States, Puerto Rico, and the Virgin Islands.

Venezuela. The campaign has been greatly intensified since 1959. Up to December 1962, of the 5,721 localities inspected in the presumably

infested areas, 577 were found positive. Of these initially positive localities, 492 were verified after treatment, and 470 were found negative in the last inspection made.

France

French Guiana. This territory has been considered negative since the 1960 reinfestation was eliminated in the same year.

Guadeloupe. The campaign was virtually suspended in 1962 because the results were unsatisfactory. A. aegypti is resistant to chlorinated insecticides, and appropriate new insecticides were not yet available.

Martinique. There has never been a specific A. aegypti eradication campaign in this island. Although the campaign conducted against insects by the local authorities continued, results with regard to A. aegypti were not satisfactory.

St. Martin. The French part of the island, administered by Guadeloupe, was considered negative. The Netherlands part, however, continued positive, which made the consolidation phase of the campaign in the French territory difficult.

Netherlands

Aruba and Bonaire. A recent verification confirmed the absence of the mosquito in these islands, which have been negative for several years.

Curacao. Eradication of the mosquito had been expected to be completed rapidly, but instead the situation worsened. The city of Willemstad and its environs are again extensively infested, and there were indications during 1962 that the vector in this area has become resistant to chlorinated insecticides.

Saba and St. Eustatius. These islands were also considered negative, but there was no recent information.

St. Martin. The Dutch part of the island was found reinfested in 1961, but administrative reasons have made it impossible to reinstate the eradication campaign.

Surinam. In 1962 an agreement providing for collaboration between the Government and the Organization was signed, and the plan of operations for the campaign was prepared. The selection and training of campaign personnel was begun in November, and eradication work proper was scheduled to begin in Paramaribo in January 1963.

United Kingdom

Antigua and Barbuda. These islands were found reinfested in 1961, and a 1962 survey of Antigua revealed extensive infestation. A. aegypti was found in 25 of the 50 localities of Antigua. The Government therefore decided to resume the eradication campaign throughout the entire territory.

Bahama Islands. The budget and the personnel of the campaign have been insufficient to deal with the problem, and the results have not been satisfactory. The susceptibility of A. aegypti to DDT is low.

Barbados. The campaign has lacked sufficient staff at its disposal; in consequence, the work cycles have been long and the results unsatisfactory. The A. aegypti in the island is resistant to DDT.

Bermuda. The island had been considered negative for several years, but the special verification to confirm eradication of the vector is yet to be made.

British Guiana. After being free of the vector for several years, British Guiana was found reinfested in 1962. The infestation was generalized in Georgetown, and it is possible that neighboring localities were also reinfested. The Government renewed operations against the vector in Georgetown but, owing to the limited number of campaign personnel and to the method employed to eliminate the mosquito, the results were not satisfactory. This situation may become dangerous because the presence of yellow fever virus was confirmed in the jungles of British Guiana in 1962.

Cayman, Turks, and Caicos Islands. The campaign has not been started in any island of this group.

Dominica. Financial reasons have prevented the Government from resuming eradication operations.

Grenada, Carriacou, and Petite Martinique. Grenada has been considered negative since 1958, but Carriacou and Petite Martinique continue positive. In Carriacou, vector resistance to chlorinated insecticides has been confirmed.

Grenadines. These islands, including Bequia and Union, continue infested.

Montserrat. The 1960 reinfestation was eliminated in 1961, but a 1962 verification showed that the island was positive again and that complete treatment is indicated.

St. Kitts, Nevis, and Anguilla. St. Kitts and Nevis continued negative, but Anguilla was highly infested. The campaign has lacked the necessary funds to intensify activities to the extent required.

St. Lucia. This island was reinfested in 1960 and again in 1961. An inspection made in 1962 showed infestation to be high and generalized. As of December 1962, financial reasons had prevented the resumption of eradication activities.

St. Vincent. This island is considered negative.

Virgin Islands. Campaign activities continued to be hampered by administrative difficulties, and results have been unsatisfactory.

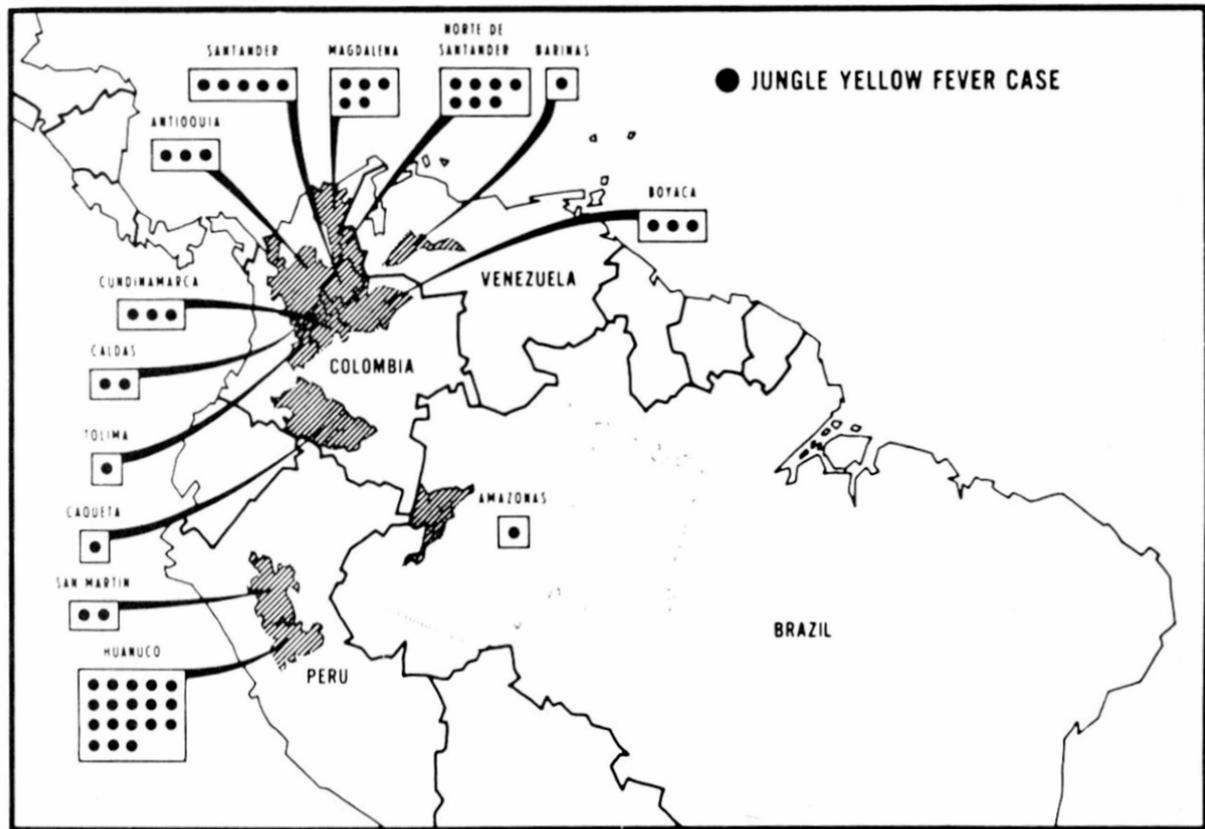


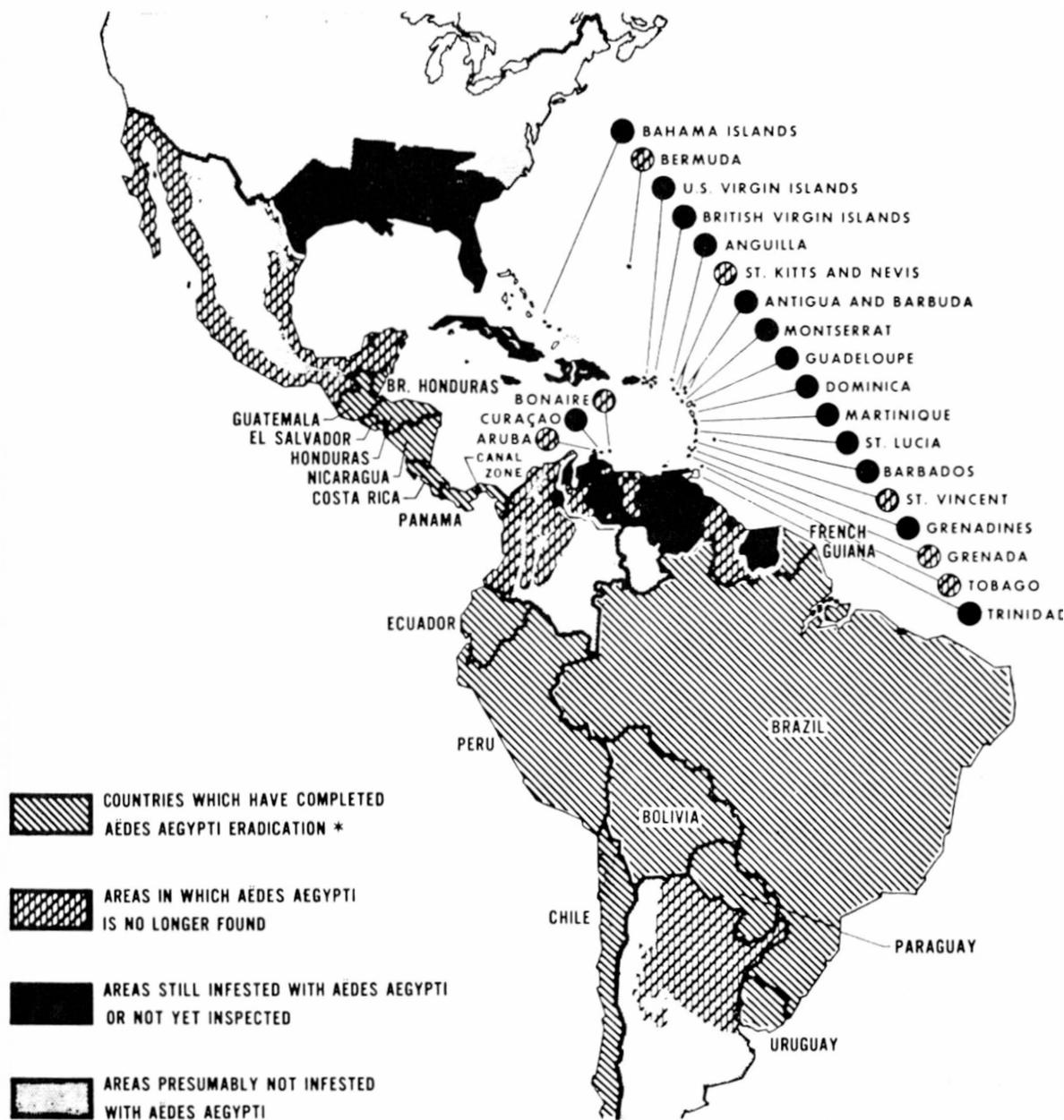
FIG. 2. REPORTED CASES OF JUNGLE YELLOW FEVER IN THE AMERICAS, 1962.

TABLE 9. USE OF YELLOW FEVER VACCINE PRODUCED
IN THE AMERICAS, 1962

Doses

Country or other political unit supplied with vaccine	Produced in	
	Brazil	Colombia
Aruba	—	3,702
Bolivia	50,000	—
Brazil	2,370,400	—
British Guiana	—	16,200
Chile	—	4,000
Colombia	—	176,915
Cuba	—	1,000
Curacao	—	2,500
Ecuador	—	1,300
Guatemala	—	10,000
Jamaica	—	1,500
Mexico	—	21,908
Panama	—	8,875
Peru	—	100,000
Venezuela	270,000	82,800
Liberia	—	3,000
Portugal	44,000	—

— None.



* ERADICATION CARRIED OUT ACCORDING TO THE STANDARDS ESTABLISHED BY THE PAN AMERICAN HEALTH ORGANIZATION

FIG. 3. STATUS OF THE *Aedes aegypti* ERADICATION CAMPAIGN
DECEMBER 1962.

TABLE 10. STATUS OF THE *Aedes aegypti* ERADICATION CAMPAIGN IN THE AMERICAS, 1962^a

Country or other political unit	Date campaign began	Date of latest available report	Area assumed initially infested		Localities inspected since beginning of campaign					Present stage of campaign
			Total	Inspected	Number	Initially positive			Still positive	
						Total	Verified			
							Treated	Number		
Square kilometers	Per cent									
Argentina	June 1953	Dec. 1962	1,000,000	100.0	3,741	165	165	165	—	b
Bolivia	June 1932	Dec. 1962	100,000	100.0	282	65	65	65	—	e
Brazil	Jan. 1931	Dec. 1962	5,358,822	100.0	268,576	36,119	36,119	36,119	—	e, d
Chile	June 1945	June 1962	104,373	100.0	301	48	48	48	—	e, d
Colombia	Nov. 1950	Dec. 1962	280,000	100.0	3,801	354	354	354	2	b
Costa Rica	April 1949	Sept. 1962	20,000	100.0	1,342	104	104	104	—	e
Cuba	March 1954	Dec. 1962	100,000	20.3	677	496	409	409	155	b
Dominican Republic	Oct. 1952	Aug. 1962	42,020	80.4	1,420	351	351	319	15	b
Ecuador	June 1946	Dec. 1962	69,454	100.0	2,824	337	337	337	—	e, d
El Salvador	April 1949	Dec. 1962	18,675	100.0	909	190	190	190	—	e, d
Guatemala	Jan. 1949	June 1962	36,423	100.0	2,485	138	138	138	—	e, d
Haiti	Oct. 1953	Sept. 1958	27,750	49.4	2,379	605	602	435	27	e
Honduras	Sept. 1949	Dec. 1962	69,929	100.0	600	53	53	53	—	e, d
Jamaica	Feb. 1950	Sept. 1962	11,424	100.0	14	12	—	—	—	e
Mexico	Jan. 1951	Dec. 1962	1,000,000	100.0	4,244	600	600	600	—	b
Nicaragua	Jan. 1950	June 1961	65,263	100.0	3,126	18	18	18	—	e
Panama	Feb. 1949	June 1960	56,246	100.0	2,853	44	44	44	—	e
Paraguay	Jan. 1948	Dec. 1962	200,000	100.0	1,561	98	98	98	—	e, d
Peru	Jan. 1940	Dec. 1962	638,000	100.0	4,320	191	191	191	—	e, d
Trinidad and Tobago	Jan. 1951	Dec. 1962	3,108	100.0	128	122	122	122	4	b
United States of America	—	—	777,000	—	—	—	—	—	—	—
Uruguay	Oct. 1948	Dec. 1962	187,000	100.0	1,020	133	133	133	—	e, d
Venezuela	June 1948	Dec. 1962	710,000	71.8	5,721	577	570	492	22	b
France										
French Guiana	May 1949	Sept. 1962	91,000	100.0	222	55	55	55	—	e, d
Guadeloupe	Jan. 1957	Oct. 1961	1,619	4.9	53	38	38	27	20	e
Martinique	Nov. 1953	March 1962	1,000	100.0	34	21	19	19	2	b
Netherlands										
Aruba	March 1952	Dec. 1962	174	100.0	9	9	9	9	—	b
Bonaire	Sept. 1952	Dec. 1962	246	100.0	6	6	6	6	—	b
Curaçao	Oct. 1951	Dec. 1962	448	100.0	5	5	5	5	4	b
Saba, St. Eustatius	July 1958	Sept. 1962	65	100.0	34	30	30	30	15	b
Surinam	Dec. 1962	Dec. 1962	48,000	—	231	74	—	—	—	b
United Kingdom										
Antigua	Aug. 1954	Dec. 1962	283	100.0	50	47	47	47	25	b
Bahama Islands	June 1954	Dec. 1962	11,396	1.3	13	11	11	11	9	b
Barbados	March 1954	Dec. 1962	171	100.0	99	98	98	98	48	b
Bermuda	Jan. 1951	Dec. 1951	53	100.0	9	9	9	9	—	b
British Guiana	March 1946	Dec. 1962	4,662	100.0	93	21	21	21	2	b
British Honduras	Oct. 1950	June 1962	22,965	100.0	84	2	2	2	—	e
Cayman Islands	—	—	259	—	—	—	—	—	—	—
Dominica	Feb. 1951	Oct. 1956	789	90.0	136	66	66	66	16	e
Grenada	Nov. 1952	July 1959	311	100.0	8	8	8	8	—	f
Grenadines	Nov. 1952	June 1962	65	100.0	7	5	5	5	4	b
Montserrat	May 1956	Dec. 1962	83	100.0	33	16	16	16	2	b
St. Kitts-Nevis-Anguilla	May 1950	March 1962	396	100.0	62	33	33	33	16	b
Saint Lucia	May 1953	Oct. 1962	259	100.0	50	50	50	50	6	b
Saint Vincent	March 1953	Dec. 1962	332	100.0	8	8	8	8	—	b
Turks and Caicos Islands	—	—	430	—	—	—	—	—	—	—
Virgin Islands	March 1960	Dec. 1962	174	74.6	23	23	23	23	8	b
United States of America										
Canal Zone	1948	Sept. 1960	1,432	100.0	21	2	2	2	—	e, d
Puerto Rico	May 1950	March 1961	8,896	61.8	481	248	248	248	116	e
Virgin Islands	—	—	124	—	—	—	—	—	—	e

— None.

. Data not available.

^a Based on reports received through 21 March 1963.^b Program in operation.^c Eradication completed.^d Vigilance continued.^e No program activity, but positive for *A. aegypti*.^f Awaiting official declaration of eradication.

REPORT FROM THE ARBOVIRUS UNIT OF THE
COMMUNICABLE DISEASE CENTER, ATLANTA, GEORGIA

Dengue in the Caribbean

Reports of epidemic dengue which appeared in Jamaica in March, in June alerted the Arbovirus Unit of the Communicable Disease Center as to the possible involvement of other areas in the Caribbean and the Aedes aegypti infested areas of the southeastern United States. Notification of the first known arrival of an acute case in the U. S. -- a 12-year-old boy who had attacks of typical illness a few hours after arrival in Miami from Jamaica on July 1 -- was given by Rockefeller Foundation staff member Dr. Wilbur Downs, from Yale University on July 24. Consultation was then established by the CDC Arbovirus Unit with Dr. Louis Grant and Dr. Edward Belle of the University of the West Indies in Kingston, Jamaica. Serological studies, using dengue types I, II, III, and IV, showed rise in titer in paired acute and convalescent sera, with highest HI and CF titer to dengue I and IV.

On request, Dr. Daniel Sudia and Mr. Craig Lyerla, arbovirus entomologist and tissue culture technologist of CDC Arbovirus Unit, were assigned to Dr. Grant's laboratory in Kingston to augment efforts to collect Aedes aegypti mosquitoes and isolate the etiological virus in human embryonic and duck embryo tissue culture, in addition to suckling mice and HeLa cells which were already being used to isolate virus from acute sera readily available from nursing staff cases in the University Hospital.

Communication established between the Arbovirus Unit of CDC and Dr. Paul Weinbren, virologist of the Puerto Rico Nuclear Center on August 20 established that epidemic dengue had been reported in Manati, about 30 miles west of San Juan, a few hours before. Collaborative studies were begun by the Puerto Rico Department of Health Laboratories and Dr. Weinbren, with assignment of Dr. Augustine Cajigas to the facilities of the Nuclear Center. Dr. Philip Coleman, in charge of the CDC Arbovirus Laboratory, dispatched dengue II antigen, goose cells, and mice by air in support of the studies which have continued to the present time.

Dr. E. L. Buescher of Walter Reed Army Institute of Research was notified in regard to the possibility of vaccine studies which

have been subsequently undertaken. At the request of the Puerto Rican Commissioner of Health, Mr. Donald Schliessman was sent on August 26, with assistance of entomologists and EIS officers of CDC to evaluate the situation in Puerto Rico. After a visit to Jamaica, Dr. T.H. Work arrived on August 28 to assist Dr. Weinbren with the serological and virological studies. Six convalescent sera were collected from patients in Manati bled eight days previously and, along with sera collected from household associates who complained of recent dengue-like illness, were set up in an HI test which showed marked rise in titer in all six pairs and high titer in most reported convalescent sera against dengue II antigen. The report was telephoned to Dr. David Sencer, Acting Chief of CDC, early in the morning of August 30. This serological diagnosis of dengue, type undetermined, established a basis for the next phase of investigation which included 1) long-term assignment of an epidemiological-entomological team under Dr. Henry Negron to provide assistance to Dr. Gonzalez of Puerto Rico in surveillance of dengue cases, 2) WRAIR vaccine studies under Dr. Buescher, referred to above, and 3) intensified efforts to isolate the etiological virus in the Puerto Rico, Jamaica, and CDC laboratories.

At this writing, all investigations are incomplete because the epidemic activity continues in both Jamaica and Puerto Rico. The disease attacks all age groups, indicating that the etiologic agent is not identical with the virus previously responsible for dengue epidemics in these two islands. Attack rates up to 50% have occurred in population groups in Puerto Rico and island-wide spread is reported. Attack rates in Jamaica have been much lower but significant.

No virus known to be the etiological agent has yet been established as a test agent in spite of intensive efforts in a multiplicity of systems in Jamaica, Puerto Rico, CDC, and armed forces laboratories. A number of cases have occurred in U.S. residents returned from visits in Puerto Rico and Jamaica. The CDC Arbovirus Unit continues to offer and provide diagnostic service in this regard and is keeping close communication with others concerned with this problem.

Eastern Equine Encephalitis-in Georgia

Excessive early summer rainfall is believed to have been a significant contributing factor to the widespread equine epizootic of eastern equine encephalitis virus transmission in the southeastern United States in 1963. Unprecedented abundance of mosquitoes was reported from many areas of the southeast.

The first equine deaths in South Georgia occurred at the end of May. In July consultations were held with Dr. John McCroan and his staff of the Georgia State Health Department in regard to an increasing number of horse cases in the region of Waycross. Collaboration was established for the virological and serological examination of equine specimens submitted from the various counties. Horse brains and sera were received from 24 of Georgia's 159 counties. Positive virological and/or serological evidence of EEE virus etiology of equine disease was established for 15 of these counties.

Table I

Virological & Serological Findings on Equine Specimens from Georgia, 1963

	<u>Month</u>	<u># Specimens Received</u>	<u># Positive</u>	<u># Negative</u>
Virological	May	1	1	0
	July	3	2	1
	Aug.	16	10	6
	Sep.	3	1	2
	Oct.	3	0	3
Total		<u>25</u>	<u>13</u>	<u>12</u>
			<u># Positive HI & CF</u>	<u># Positive HI Neg. CF</u>
Serological	June	3	0	0
	July	1	0	1
	Aug.	10	2	7
	Sep.	4	0	1
	Oct.	3	0	1

Because of the interest and investigations by the state and local health authorities, the continuous provision of information and specimens outlined an epizootic situation which might have developed into a fall EEE epidemic in man. Two additional facets were consequently investigated: 1) serological evidence of any EEE infection in the horse associated farm residents, and 2) intensive mosquito collection on the epizootic premises to determine whether any vectors other than non-human biting Culiseta melanura were infected.

Epidemiology Branch of CDC assigned Dr. Myron Schultz, an Epidemiological Intelligence Officer, to the Arbovirus Unit for one month to undertake serological collections from the equine case associated human farm residents of southeastern Georgia. Of 312 human sera collected in the epizootic area, only six were HI positive to a standard EEE antigen and a hemagglutinin made from a recent equine EEE isolate, in addition to antigens for WEE and SLE viruses. There was no significant difference in the reactivity of the two antigens. The ages of the EEE positive serum donors were: 86/F, 79/F, 56/M, 50/F, 25/F, and 17/F. None had appreciable titers of complement fixing antibody which indicated that the infections were not recent.

While these EEE positive sera indicate probable past infection with EEE virus, unassociated with overt CNS disease, it was not very significant as an indication that EEE virus was currently being transmitted to human residents of the epizootic area. It is evident from the table that equine deaths subsided by the end of September, an important reason being that most of the remaining animals had been vaccinated with EEE vaccine by the end of August.

This decline in equine disease as a sentinel reflection of EEE virus activity increased the significance of the weekly and biweekly mosquito collections initiated at the beginning of August by Dr. Chamberlain, Dr. Sudia, and Mr. Johnston of the Arbovirus Vector Laboratory of the CDC Arbovirus Unit. Table II summarizes to date the results of examining about three-fourths of the 2001 mosquito pools collected in the three month period, July-October.

Table II

Mosquitoes Collected near Waycross, Ware County,
Georgia, 1963, for Virus Tests

	8/2-4	8/7-9	8/14-16	8/21-23	9/5-7	9/18-20	10/8-11	Total
<i>Aedes</i>								
<i>atlanticus</i>	3358 (84)*	3409 (77)	1981 (49)	1100 (28)	2900 (63)	283 (14)	28 (11)	13059 (320)
<i>canadensis</i>	87 (16)	36 (8)	19 (8)	15 (6)	12 (7)			169 (45)
<i>dupreii</i>	179 (20)	68 (9)	131 (9)	44 (7)	81 (12)		1 (1)	504 (58)
<i>fulous pallen</i>	3 (2)	4 (2)						7 (4)
<i>infirmatus</i>	201 (14)	256 (17)	4 (2)	2 (2)	11 (5)	1 (1)		475 (41)
<i>mittchellae</i>	228 (16)	575 (19)	152 (13)	176 (12)	100 (8)	16 (7)	2 (2)	1249 (77)
<i>triseriatus</i>	14 (7)	15 (7)	8 (3)	2 (1)	3 (3)	1 (1)	1 (1)	44 (23)
<i>vexans</i>	26 (12)	42 (11)	28 (10)	24 (9)	9 (4)	3 (3)	17 (10)	149 (59)
<i>Anopheles</i>								
<i>crucians</i>	2913 (43)	2799 (40)	1335 (18)	2666 (40)	2301 (46)	658 (16)	278 (15)	12950 (218)
<i>punctipennis</i>			1 (1)			1 (1)		2 (2)
<i>quandrimaculatus</i>	9 (6)	4 (3)	19 (7)	18 (8)	5 (4)	31 (8)	6 (4)	92 (40)
<i>Culex</i>								
<i>nigripalpus</i>	225 (21)	210 (16)	639 (21)	1182 (21)	414 (15)	654 (14)	378 (12)	3702 (120)
<i>quinquefasciatus</i>	2 (2)	2 (1)	1 (1)			2 (2)	9 (5)	16 (11)
<i>restuans</i>		1 (1)				2 (2)	15 (9)	18 (12)
<i>salinarius</i>	150 (14)	134 (11)	35 (6)	72 (9)	57 (9)	23 (9)	53 (8)	524 (66)

	8/2-4	8/7-9	8/14-16	8/21-23	9/5-7	9/18-20	10/8-11	Total
territans	6 (4)	2 (2)	7 (2)	3 (3)		1 (1)	5 (4)	24 (16)
(Mel) sp.	375 (22)	207 (16)	220 (15)	446 (14)	247 (13)	58 (10)	1 (1)	1554 (91)
Culiseta melanura	3153 (75)	2849 (63)	3118 (68)	5466 (114)	2019 (43)	541 (16)	366 (23)	17512 (402)
Mansonia perturbans	65 (17)	33 (11)	23 (11)	15 (8)	30 (9)	9 (7)	1 (1)	176 (64)
Orthopodomyia signifera						1 (1)		1 (1)
Psorophora ciliata	66 (15)	754 (31)	224 (16)	89 (8)	71 (11)	26 (7)	3 (3)	1233 (91)
confinnis	1004 (25)	1121 (22)	270 (13)	399 (14)	272 (14)	66 (9)	33 (7)	3165 (104)
discolor	6 (3)	1 (1)	1 (1)					8 (5)
howardii	1 (1)	5 (4)	2 (2)					8 (7)
ferox	16 (8)	16 (6)	20 (6)	1 (1)	3 (2)	1 (1)		57 (24)
Uranotaenia lowii	4 (3)	11 (1)		1 (1)	1 (1)			17 (6)
sapphirina	374 (15)	410 (16)	1042 (12)	990 (12)	303 (10)	290 (11)	74 (12)	3483 (88)
Total mosquitoes	12,465	12,964	9280	12,711	8839	2668	1271	60,209
No. trap nights	180	120	116	94	102	116	168	896
#Mosq/trap night	69	108	80	136	86	23	7	67
Total pools tested	445	395	294	318	279	141	129	2001

*No. in parenthesis = No. of pools

It appears that the heavy precipitation of early summer was compensated by a record dry spell from late September to November with resulting rapid decline of mosquito activity which may have inhibited the speculated EEE epidemic in the cooler weather of late fall.

As so often happens in the investigation of specific problems such as the EEE epizootic in south Georgia, dividends of an unexpected nature result. The large number of other arbovirus isolates recovered from this study will be reported on later when the isolations are complete, characterized, and identified.

Table III

Viruses Isolated from Mosquitoes Collected near Waycross,
Ware County, Georgia, 1963

Virus	Mosquito species	8/2-4	8/7-9	8/14-16 [*]	8/21-23 [*]	9/5-7 ^{**}	9/18-20	10/8-11	Sub total	Grand total
EEE	C. melanura	13	7	1	1		1	1	24	26
	A. atl-torm.	1							1	
	C. nigripalpus		1						1	
Other viruses	A. atl-torm.	16	6	1			2		25	<u>70</u> 96
	C. melanura	7	8	1	4				20	
	P. confinnis	5	7				1		13	
	An. crucians	6	1		1				8	
	An. quad.	1							1	
	A. mitchellae		3						3	

*Only a part of the mosquitoes collected have been tested.

**None of the mosquitoes collected have been tested.

REPORT FROM DRS. A. D. HESS, L. C. LAMOTTE, ..
PRESTON HOLDEN, AND D. BRUCE FRANCY OF THE
DISEASE ECOLOGY SECTION, USPHS
COMMUNICABLE DISEASE CENTER, GREELEY, COLORADO

On July 1, 1963, the activities of the CDC Encephalitis Section at Greeley, Colorado, were expanded to form a new Disease Ecology Section within the Technology Branch. The primary function of the Disease Ecology Section is to conduct research on the ecology and control of encephalitis, plague, tularemia, Colorado tick fever, and other selected communicable diseases which are prevalent in the western United States. Greeley remains the headquarters of the new section, and the principal field stations are the encephalitis station at Bakersfield, California, and the plague station at San Francisco, California. The Taunton, Massachusetts, field station has been transferred to the Virology Section, Laboratory Branch, CDC, Atlanta, Georgia. Dr. A. D. Hess is Chief of the new Disease Ecology Section. Dr. Preston Holden has joined the staff of the section as research virologist.

Arbovirus Activity, 1962

Very little WE or SLE virus activity was apparent in Colorado during 1962. HAI rates for either virus were low and no virus isolations were made from mosquitoes. Sentinel chickens in northern Texas had HAI rates of 62 to 86 per cent to WE antigen and SLE rates of 0 to 11. Virus isolations from Texas mosquitoes indicate an infection rate of one per 1,000 for WE and zero per 1,000 for SLE, down considerably from previous years. Serologic evidence also suggested little WE or SLE in the Quincy, Washington, area.

The discrepancy between HAI rates when several WE virus strains were used as antigens, found in the 1961 Colorado sentinel chickens, was not so pronounced in 1962. As in 1961, the Texas chicken sera had a uniform pattern of reaction; every specimen which had an inhibitor for one strain of WE almost invariably had an inhibitor for the other WE strain used. Very few of the Washington State chickens had inhibitor to one but not to the other WE virus strain.

The time of virus activity in northern Texas, as indicated by HA inhibitors in interval-bled flocks, was between mid-August and mid-September. SLE inhibitor was frequently not detected in sera collected in early October, but was detected in those sera collected at the end

of October. This suggests either a delayed antibody formation or that SLE is transmitted as late as mid-October.

As in the past three years, 1962 Taunton, Massachusetts, specimens gave evidence only of WE activity. HAI and neutralization tests indicated that only WE virus was active in sentinel chickens, and only WE virus was isolated from mosquitoes. Twenty-three per cent of the 180 chickens had an HA inhibitor to WE McMillan antigen, and 19 per cent had neutralizing antibody. Three WE isolates were obtained from 194 pools of arthropods, mostly mosquitoes. One of these isolates was from a pool of Culex pipiens.

The WE viruses isolated from northern Texas in 1962 gave uniform plaques of 4-5 mm in DETC, as did the Taunton, Massachusetts, WE isolates. In plaque-reduction neutralization tests, it was noted that the same WE immune serum run simultaneously against the Texas and Taunton WE isolates gave 83 per cent to 95 per cent plaque reduction. This WE immune serum also apparently caused an inhibition of plaque size among the Texas isolates. The plaques were 1 mm in diameter in the WE immune serum bottles, and 4-5 mm in diameter in the SLE and EE bottles. However, this serum did not inhibit plaque size of the Taunton isolates. This is of interest in view of the recent data suggesting certain differences between WE virus strains.

Encephalitis in Texas Panhandle

An outbreak of encephalitis currently in progress in Hale County, Texas, and adjacent counties in the High Plains area is now under investigation. In cooperation with the Texas State Health Department and the Hale County Health Department, efforts are being made to determine the extent of the outbreak and the etiologic agent or agents responsible.

As of 30 August, 50 human cases of suspected encephalitis had occurred in Hale County, which has a population of around 50,000. Fourteen suspected horse cases had been reported in the same area. The first reported human case occurred during the fourth week of June. The greatest number of suspected cases, as of the end of August, occurred during the week of 12-18 August, totaling 19 in number. Both human and horse cases were reportedly seen by physicians and veterinarians in surrounding counties. Two human deaths were associated with the outbreak in Hale County, and three additional deaths were reported from

the surrounding areas. Physicians reported a high frequency of a syndrome usually featuring severe frontal headache, nuchal rigidity, fever, nausea, and pleocytosis. Approximately 30 per cent of the suspected cases were in children under 5 years of age.

Based upon light trap collections up to mid-July, it appeared that C. tarsalis populations were somewhat higher than during 1962, with high indices occurring 2-3 weeks earlier.

Viral agents have been isolated from 1 of 5 human cerebrospinal fluids, 44 of 71 pools of C. tarsalis, and 1 of 3 pools of C. quinquefasciatus collected in Hale County. The spinal fluid isolate and the 11 mosquito isolates identified have proved to be WE virus. HAI tests on 24 unpaired human sera has shown 4 patients to have inhibitor to the WE viruses but not for SLE.

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

In the March 1963 issue of Arthropod-borne Virus Information Exchange, we reported the presence of HI antibody to EE virus in several chicken sera collected in the Tucson area. Dr. Carl M. Eklund, of the Rocky Mountain Laboratory, Hamilton, Montana, kindly agreed to do neutralization tests on these HI positive sera. These neutralization tests in mice demonstrated no neutralizing antibody for EE virus. Our results are similar, therefore, to those reported by Drs. Goldfield and Sussman, of the New Jersey Department of Health, and by Dr. Daniels, of the Massachusetts Department of Public Health, in the October 1962 issue of Arthropod-borne Virus Information Exchange. It is not clear whether this HI antibody is due to an infection with a virus serologically related to EE or whether it is non-specific in nature.

Further studies have been performed to confirm the identity of a virus isolated from a pool of Culex quinquefasciatus captured in the environs of Tucson in October 1962. A hemagglutinin has been prepared with this virus, designated A-169, by the sucrose-acetone method, and hyperimmune sera have been produced in rabbits and mice. Neutralization tests in both suckling and 3-week-old mice indicate that the A-169 virus is closely related to SLE.

Sera collected from Navaho Indians (mostly adults) in north-western Arizona have been tested as follows:

Cache Valley Virus - HI test - 2/55 positive results.

Colorado Tick Fever - Complement fixation test - 0/254 positive result.

In June, mosquito collections in the environs of Tucson were begun. It will be noted from the data below that few mosquitoes were captured until some days after the beginning of the summer rainy season, about the middle of July:

	<u>Culex</u> <u>quinquefasciatus</u>	<u>Culex</u> <u>tarsalis</u>	<u>Psorophora</u> <u>confinnis</u>
June 16-30	175	0	0
July 1-27	10	0	0
July 28-August 10	555	0	44
August 11-24	4132	118	35

The relative scarcity of Culex tarsalis is noteworthy and confirms the experience of the 1962 hot season. These mosquitoes are being tested for the presence of virus by inoculation into suckling mice and by culture in hamster kidney cells.

Collections of ticks are also being made, both from wild animals and occasionally on the Indian reservations from humans. These also are being tested for virus.

REPORT FROM DRS. J. V. IRONS, J. S. WISEMAN, AND JULIAN FIELD
STATE DEPARTMENT OF HEALTH, AUSTIN, TEXAS

In 1962, a few sentinel flocks of chickens were distributed for determining virus activity in certain areas of the state. Of 133 sera obtained from Orange County in southeast Texas, 10% were reactive for EE only or EE and WE. (In Exchange #7, Orange County findings had been erroneously reported negative). More varied results were obtained on the 86 sera taken from sentinel chickens in El Paso, but with only slight evidence of SLE activity. The low level of SLE activity was in

line with lack of SLE infection in man. Seven WE human infections had serological confirmation. Six of 7 cases were from the high plains area.

Grimes and Coleman have evidence which suggests that several if not all the unidentified viruses remaining from 1962 mosquito pools perhaps are Hart Park related.

So far this year, 2774 adult mosquitoes have been received for virus isolation studies, from Orange, Bowie, El Paso, Lubbock, and Wichita Counties. Most areas of the state have experienced an unusually dry year and mosquito populations have been abnormally low. The mosquitoes have been processed in a total of 83 pools. Virus isolates have been obtained from seven pools of Culex tarsalis mosquitoes from the Lubbock area, three of which have been identified as WE virus.

The finding of virus in mosquito pools is of interest in view of an upsurge of clinical cases of encephalitis in the high plains of northwest Texas. Of the few that have yet been tested, only 2 WE cases have received confirmation by mid-August. This year, several sentinel flocks of chickens were placed in several areas of the state, particularly along the Gulf Coast.

REPORT FROM THE USAF EPIDEMIOLOGY LABORATORY
LACKLAND AFB, TEXAS, DIVISION OF EPIDEMIOLOGY

The occurrence of the July-September 1962 outbreak of viral encephalitis in the Tampa Bay area of Florida, partially documented to have been due to St. Louis encephalitis virus, has been adequately described in previous issues of this Exchange (No. 6, October 1962, and No. 7, March 1963). It was pointed out that relative absence of SLE epidemic infections in Hillsborough County, adjacent to other areas where peak infections occurred, remained unexplained.

MacDill Air Force Base, Florida, is located on a peninsular extension of Hillsborough County into the northern portion of Tampa Bay. The existence of an easily studied, presumably exposed population of military personnel and civilian dependents residing and working on base, offered an opportunity to study factors which may

have been in part responsible for the comparatively low incidence of epidemic infections in the Hillsborough County area.

Clinical cases of suspected viral encephalitis among MacDill hospitalized patients numbered eight during the period of the 1962 outbreak, although laboratory confirmation of SLE infection was obtained on only three of these patients. Thus, an attack rate of 2.5 laboratory confirmed cases per 10,000 population served by the MacDill hospital was observed. Of these, all had visited, resided, or had been employed in the St. Petersburg-Clearwater area (Pinellas County) at times which would have been appropriate for acquiring their infections in this area. All confirmed cases occurred in adults ranging in age from 29 to 57.

In early September 1962, approximately two weeks following the peak of encephalitis infection in Pinellas County and at the time of the much less impressive peak of infection in Hillsborough County, sera were collected from a random sample of military and dependent personnel, comprising roughly 4% of the total eligible population serviced by the 836th TAC Hospital at MacDill AFB. Follow-up sera were obtained on approximately one-half of the original sample in late October 1962. Paired specimens were thus obtained from 132 children (below age 16) and 109 adults.

Results of hemagglutination inhibition tests, utilizing four arbovirus antigens, are shown in the following table.* Antigens include (1) the TBH strain of St. Louis encephalitis virus (SLE), (2) the NJO strain of Eastern encephalitis virus (EE), (3) the SN66 strain of Highlands J virus (HJ), and (4) the newly recognized Tensaw virus, strain A-9-171b.

Age Group	Number Individuals Tested	HI Tests Positive ^a at 1:10 dilution or greater with							
		SLE		HJ ^b		EE		Tensaw	
		No.	Rate (%)	No.	Rate (%)	No.	Rate (%)	No.	Rate (%)
<16	132	0	0	2	1.5	0	0	2	1.5
>16	109	5	4.6	1	0.9	0	0	5	4.6
Total	241	5	2.1	3	1.2	0	0	7	2.9

^a 1st, 2nd, or both serum specimens positive.

^b All specimens positive to HJ antigen were likewise positive at equal or greater titers to Western encephalitis virus (Fleming strain).

It is of interest to note that all sera positive by HI test with SLE antigen were derived from adult males, all enlisted men aged 23-41, only one of whom had received previous yellow fever immunization. Serum neutralization tests are being conducted on the HI-positive sera to determine specificity for SLE virus.

Previous testing of sera collected from a random sample of the general population of Hillsborough County by the CDC and TABREL laboratories indicated an overall infection rate by SLE or a closely related antigen in the order of 4%. The reduced rate of roughly 2% in the MacDill population may have been due to any of several factors. The sera from MacDill AFB had been stored in excess of eight months, and antibody loss may have occurred. The MacDill sample, by virtue of the population studied, was devoid of any specimens from elderly individuals, in whom clinical infection rates were highest. Many of the individuals included in the sample from MacDill AFB were transferred to that base subsequent to the previous exposures to SLE virus that occurred in the 1959 and 1961 Tampa Bay epidemics.

During the period immediately prior to and during the Tampa Bay epidemic of 1962 mosquito light trap counts from housing, work, and school areas on MacDill AFB revealed that Culex nigripalpus, the suspected epidemic vector, comprised approximately 2% of the total mosquito populations. Daily insecticide fogging operations during the summer and fall were apparently effective in significantly reducing all but salt marsh mosquitoes. Aedes taeniorhynchus comprised approximately 95% of the total mosquito counts from light traps. During the period June-October 1962, total light trap counts were reduced roughly 90% from the average levels experienced in these areas during comparable periods of 1957-1959. In fact, insufficient numbers of Culex mosquitoes could be collected in September and October 1962 to allow for attempted viral isolations. Such reduction in Culex populations may have been responsible for lowered infection rates.

Two viral isolates were obtained from pooled A. taeniorhynchus collected in mid-September 1962 from MacDill AFB. One of the agents has been identified by serum neutralization tests in suckling mice as SLE virus. The isolate has been sent to the CDC Arbovirus Laboratory for confirmation. Although this vector is known to be capable of laboratory infection and transmission by bite, isolation of the agent from naturally infected A. taeniorhynchus has not previously been reported.

The second isolate, tentatively designated EPE 93, appears to be closely related to or identical with the Tensaw virus of the Bunyamwera group. Guinea pig antiserum to EPE 93 neutralized 2.9 logs of Tensaw virus and 3.4 logs of homologous virus. Hemagglutination, which occurred optimally at pH 6.0-6.2 at 37°C or room temperature, was not inhibited by SLE, EE, WE, or CEV antiserum. Adult and weanling mice were susceptible to intracranial inoculation of the agent but were insusceptible by the intraperitoneal route. Half-day-old chicks were insusceptible by both the intradermal and intracranial routes. Confirmation is presently being attempted by Dr. W.McD. Hammon, University of Pittsburgh.

*We are indebted to Drs. Telford Work and P.H. Coleman and to their staff members of the Virology Section of CDC, Atlanta, for providing technical training of and facilities for the conduct of these tests by Miss Janet Twitchell of the USAF Epidemiology Laboratory.

REPORT FROM DR. S. S. KALTER
SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION
SAN ANTONIO, TEXAS

A field trip to Darajani, Kenya, is now underway for the purpose of collecting bloods, stools, and throat washings on a representative portion of the human population and approximately 100 to 200 baboons. These specimens are to be tested for various viruses prevalent in man. Because the pilot study on baboon antibodies to the arboviruses, as reported in a previous issue of the Arthropod-borne Virus Information Exchange, indicated little evidence of arbovirus infection, this study will be repeated employing larger numbers of animals and including sera from the native population.

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

The idea that the bat could be involved in the epidemiology of yellow fever is not new. Kumm, in 1932, having observed that mosquitoes fed on various species of bats in the laboratory, attempted transmission studies using red mastiff bats and vampire bats.

Although this and subsequent studies by other investigators (Rodhain, 1936; Laemmert et al, 1946) on experimental yellow fever infection in bats failed to provide evidence that these animals could be infected with this virus by inoculation or by the bites of infected mosquitoes, an evaluation of the experimental design of these early studies suggests that Kumm's original hypothesis has not received adequate testing. Because of the difficulties involved in maintaining bats in the laboratory, the early studies were carried out with small numbers of animals tested over short periods of time and conclusions were based on attempts to isolate virus from the blood or to demonstrate specific neutralizing antibody. In no instances were tissues other than blood tested.

In experiments previously reported in Information Exchange No. 5, it has been shown that species of insectivorous bats are susceptible to certain strains of JBE and SLE viruses. As part of studies aimed at determining if the bat may act as a reservoir host for the arboviruses, it seemed of value to re-investigate the susceptibility of bats to yellow fever virus. During the course of a preliminary study in which big brown bats (Eptesicus f. fuscus) received 1000 mouse intracerebral LD₅₀ of the 17D strain of yellow fever virus subcutaneously, virus was found in the blood, brown fat, spleen and brain of one adult bat sacrificed 18 days after inoculation, and in the brown fat tissue only of two young bats sacrificed 4 and 7 days, respectively, after receiving virus.

Since the susceptibility of various bat species to JBE and SLE viruses was found to vary with the strain of virus used (Am. J. Trop. Med. and Hyg., September, 1963), additional pilot experiments were initiated to determine the susceptibility of the Mexican free-tailed bat (Tadarida b. mexicana) to the French neurotropic strain of yellow fever virus obtained from Dr. Max Theiler. Tadarida appear to be much more susceptible to this strain of yellow fever than are the big brown bats to the 17D strain. Virus could be demonstrated in the blood, brown fat, brain, kidney, and spleen of bats beginning 7 days after subcutaneous inoculation of virus and persisted in the blood of some animals for at least 14 days. At no time were signs of encephalitis observed in these animals.

These results indicate that at least two species of insectivorous bats are susceptible to yellow fever virus and could be involved in the natural history of this disease. Failure of early workers to obtain firm evidence of the multiplication of yellow fever virus in the bat was due

possibly to the inability to maintain these animals in the laboratory in a healthy condition for extended periods and to the fact that bat species may vary in susceptibility to various strains of yellow fever virus. These studies are being continued in an effort to evaluate the role Chiroptera may play as a reservoir host for yellow fever.

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

TICK TRANSMITTED VIRUSES

Colorado Tick Fever (CTF)

I. HUMAN DISEASE

This summer virus has been isolated from the blood of 37 patients. The maximum incidence of disease is usually during the months of May and June. This year the maximum incidence occurred in June, but the case rate in both May and July was also relatively high. The delay in the appearance of the disease and the prolonged occurrence may be attributed to cold weather.

Seasonal Incidence of CTF

	March	April	May	June	July	August	Sep.	Oct.	Total
1963*		1	8	17(1)	9	(1)			37
1948-1962	6	69	185	227	121	20	2	1	631

*Through 27 August 1963.

Figures in parentheses = isolates with incomplete identifications to date.

Only one patient was of interest from the clinical standpoint. A 42-year-old man was suspected of having encephalitis on the basis of severe headaches of several days' duration.

No cases were recognized outside the previously known geographical distribution.

II. FIELD OBSERVATIONS ON CTF VIRUS

a) Amount of virus in adult Dermacentor andersoni. D. andersoni collected in one of the canyons of the Bitter Root Valley were ground in pools of 10, and serial dilutions were inoculated intraperitoneally into 3-4-day-old mice. The table shows the dilution in which virus was detected and the approximate amount of virus present.

Amount of Virus in Pools of 10 Adult D. andersoni

Diln. of tick suspension	10^{-4}	10^{-3}	10^{-2}	10^{-1}	No virus detected
Approx. amount of virus	$10^{4.2}$	$10^{3.2}$	$10^{2.2}$	$10^{1.2}$	$< 10^{1.2}$
No. of pools with virus in indicated dilution	1	5	4	6	5

The amount of virus present in adult ticks is very low or the methods of detecting virus are very insensitive. In experimentally infected D. andersoni, similar amounts of virus are also found. By contrast, in Culex tarsalis the amounts of virus in the naturally infected mosquito are in the range of one-half to one million suckling mouse LD₅₀.

b) Time of activity of larval and nymphal D. andersoni. During the past 2 years in the Black Hills of South Dakota, larvae and nymphs of D. andersoni have been collected during the months of May, June, July, and August. Virus has been isolated from the blood of a chipmunk and from nymphs as early as May 7th, and from nymphs and small mammals during the rest of the summer.

These data indicate that there is a nymph-small mammal cycle operating at least during these 4 months.

The relative proportion of larval and nymphal D. andersoni appears to change during the summer. For instance, 83 larvae and 137 nymphs were collected during May, 1963, whereas 432 larvae and 19 nymphs were collected during July, 1963. Whether the varying proportion of larvae and nymphs is significant in the maintenance of CTF virus is not clear.

To date, isolation of virus from small mammals has been more frequent in late summer, but more study is needed before one can state that one part of summer is more favorable for virus isolation than another.

c) CTF virus in D. variabilis. This year 1124 D. variabilis from North Dakota and Minnesota were examined, and no virus was isolated.

To date, 16,670 D. variabilis have been examined with no isolation of any virus in suckling mice.

III. LABORATORY OBSERVATIONS ON GROWTH OF CTF VIRUS IN D. ANDERSONI

Using another strain of CTF virus, Rush has confirmed the finding of Kennedy, previously reported, that the largest amount of virus is found during the nymphal stage. Rush has also shown that when nymphs engorge on infected hamsters virus first appears in the salivary glands of the nymphs 11 days after feeding was completed. When larvae engorge on infected hamsters, virus first appears in the salivary glands 15 days after feeding was completed. The engorged larvae molt to the nymphal stage before the virus appears in the salivary glands. There appears to be an extrinsic incubation period in ticks infected with CTF virus comparable to that seen in mosquitoes infected with viruses such as that of yellow fever.

POWASSAN VIRUS

This year two isolations of virus have been made from 334 larvae and nymphs of Ixodes spinipalpus from Peromyscus, an isolation rate that compares favorably with that found for CTF virus in engorged D. andersoni larvae and nymphs. The time of occurrence of I. spinipalpus larvae and nymphs differs somewhat from that of D. andersoni. I. spinipalpus is primarily an early summer ectoparasite, although its occurrence overlaps that of D. andersoni such that larvae and nymphs of both species are often found on the same animal. This may account for the occasional occurrence of Powassan virus in D. andersoni and of CTF virus in Ixodes spinipalpus.

Antibodies have been found in sera obtained from chipmunks, field mice, deer mice, and jumping mice.

Neutralizing Antibodies in Sera from Small Mammals in Black Hills, S. D.

	1961			1962		
	No.	No. +	%	No.	No. +	%
Chipmunks (<i>Eutamias</i>)	63	4	6	56	5	9
Field Mice (<i>Microtus</i>)	12	2	18	101	13	13
Deer Mice (<i>Peromyscus</i>)	50	1	2	83	23	28
Jumping Mice (<i>Zapus</i>)				9	2	22

It is concluded that *I. spinipalpus* is an important vector of Powassan virus in the Black Hills on the basis of the isolation of virus from that species, the frequent infestation of small mammals with that species, and the finding of antibodies to the Powassan virus in these mammals. Since *I. spinipalpus* is rarely found on man, human infection seems likely to occur only from the bite of the occasional *D. andersoni* that becomes infected from the *I. spinipalpus* cycle, and it is not anticipated that Powassan virus will prove to be a public health problem in the Black Hills. Sera from 174 people who live in the Black Hills and who frequent the canyon where Powassan virus was isolated have been checked for Powassan antibodies. No antibodies were found, however, which provides additional evidence that Powassan virus is not a public health problem in the Black Hills.

MOSQUITO TRANSMITTED VIRUSES (WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS)(WEE & SLE)

In the search for an overwintering mechanism for these viruses, the hypothesis that there are mechanisms independent of mosquitoes is being explored as part of this study. An attempt is being made to determine whether there is any relation between antibody incidence in mammals and the occurrence of virus in *Culex tarsalis*. Last year there was only one isolation of WEE virus from *C. tarsalis* and five of SLE virus, yet there was evidence of WEE antibodies (hemagglutination inhibition - HI) in deer mice, field mice, the common house mouse, and SLE antibodies (HI) in the sera of these species and in harvest mice. Chicken and hog sera showed no SLE antibodies to these viruses. There were no WEE antibodies in the chicken sera, and a relatively low incidence in the hog sera.

HI Antibodies in Animal Sera Collected in Eastern Oregon During
Winter and Spring of 1963

	WEE Antibodies		SLE Antibodies	
	February	May	February	May
Peromyscus	3/46 (6.5%)	3/147 (2%)	9/46 (19.5%)	26/147 (17.7%)
Microtus	14/97 (14.4%)	0/76 (0%)	2/97 (2%)	4/76 (5.2%)
Mus musculus	0/42 (0%)	1/30 (3.3%)	12/42 (28.6%)	1/30 (3.3%)
Reithrodontomys		0/16		2/16 (12.5%)
Hog sera (collected Sep 62)	3/44 (6.8%)		0/44 (0%)	
Chicken sera (collected Sep 62)	0/50 (0%)		0/50 (0%)	

These data give no evidence of group A activity during the winter, but they do suggest that there may have been group B infection in deer mice. The appreciable WEE infection in field mice during the summer of 1962 and the extensive group B infection in deer mice and the common mouse appears out of proportion to the amount of mosquito activity found.

Attempts at isolation of virus from the brain and spleen of 91 deer mice, 113 field mice, 60 common mice, and 2 shrews collected during February, 1963, have been negative.

In the study area in eastern Oregon, C. tarsalis has not appeared in any numbers during the summer of 1963, apparently because of cool weather. Attempts at isolation have not yet been made.

With mosquito activity at a minimum during the summer of 1963, there should be an opportunity to demonstrate whether infection in small mammals is independent of mosquito activity.

In North Dakota during the summer of 1962, no virus was isolated from over 3000 C. tarsalis collected in midsummer yet WEE antibodies were demonstrated by HI in 31 of 50 hog sera (62%) suggesting either that WEE infection in C. tarsalis was focal in nature or hogs became involved in a cycle independent of C. tarsalis.

REPORT FROM DR. NORMAN D. LEVINE, ACTING DIRECTOR,
CENTER FOR ZONOSSES RESEARCH,
UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS

This center was activated late in 1960. Its director is Dr. Carl A. Brandly, Dean of the College of Veterinary Medicine, University of Illinois, and its associate (scientific) director is Dr. LeRoy D. Fothergill, Scientific Advisor, U. S. Army Biological Laboratories, Ft. Detrick, Maryland. Dr. Robert H. Kokernot, formerly of the Rockefeller Foundation, joined the center as assistant director in August 1963.

Senior members of the center include members of the University Departments of Anthropology, Dairy Science, Entomology, Geography, Physiology, Preventive Medicine, Veterinary Medical Science and Zoology, the University Health Service, the Illinois State Natural History Survey, the Illinois Department of Public Health, and the Quadri-County Department of Public Health.

Consultants include members of the Division of Water Supply and Pollution Control, Department of Health, Education, and Welfare, Cincinnati, Ohio; the Pan American Health Organization, World Health Organization, Washington, D. C. ; the U. S. Army Chemical Corps Biological Laboratories, Ft. Detrick, Maryland; the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; the Naval Medical Research Unit No. 4, Great Lakes, Illinois; the Rockefeller Institute, New York City; the Communicable Disease Center, U. S. Public Health Service, Atlanta, Georgia; the U. S. Armed Forces Institute of Pathology, Washington, D. C. ; the Plum Island Animal Disease Laboratory, Long Island, New York; and the Gorgas Memorial Laboratory, Panama.

The first field study to be carried out by the center has been initiated with the aid of a grant on the emergence and recession of the zoonoses from the U. S. Public Health Service, administered through the Communicable Disease Center. A coordinated zoonotic observational network has been established in the Quadri-County area (Massac, Johnson, Pope, and Hardin Counties), of southern Illinois. Field headquarters have been established at Metropolis, Illinois, and more than 30 persons have been engaged in the work, with Dr. William C. Marquardt as Field Coordinator.

Emphasis is being given to studies on the emergence and recession of arthropod-borne virus encephalitides. Virus isolation and serologic studies are being coordinated with studies of the physical geography, demography, climatology, ecology, entomology, parasitology, and human and animal epidemiology of the region. All information is being transferred to IBM cards and will be analyzed by the IBM 7090 digital computer. However, at the time this report was prepared, insufficient information had been analyzed to justify a discussion of results. This will be the subject of subsequent reports, and the purpose of the present one is simply to inform other investigators that this research has been initiated.

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

Tests have been completed on 87 vertebrates either shot or trapped within a 20-mile radius of Powassan, Ontario, between 1st and 24th May 1963. Neutralizing antibody to Powassan virus was detected as follows:

Incidence of Powassan neutralizing antibody in forest mammals, Powassan Area, May through July 1963

Species	Antibody incidence	Ixodes sp. infestation	Township of highest Infection rate
Squirrel (<i>Tamiasciurus hudsonicus</i>)	12/102*	3/24 **	Nipissing
Woodchuck (<i>Marmota monax</i>)	15/29	4/7	Bonfield, Nipissing
Porcupine (<i>Erethizon dorsatum</i>)	10/30	0/2	Nipissing
Snowshoe hare*** (<i>Lepus americanus</i>)	0/91	HLP	
Others: mice, voles, etc.	0/80	0/3	
Totals	37/332	7/36	

*Ratio of animals with antibody to those tested.

**Proportion of tick-infested animals which had antibody.

***23 of 76 hares had Silverwater antibody.

None of the tick pools or blood samples have yielded virus by inoculation of suckling mice intracerebrally.

REPORT FROM JOAN B. DANIELS
VIRUS SECTION, DIAGNOSTIC LABORATORIES
MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH
BOSTON, MASSACHUSETTS

Clinical western encephalitis has not been recognized in Massachusetts. Evidence for a single "silent" infection was found (neutralizing antibody) in one out of 328 human bloods collected in 1961. Again in 1962, as in each year since 1959, there was much evidence of western encephalitis activity in sentinel chickens and wild birds but no overt disease observed. Clear evidence of eastern encephalitis activity in 1962 has not been found; mosquito collections are still to be examined. This is an interesting reversal of incidence of the two viruses. In this section of the country, the ecology of the two viruses may be very similar since they have been found almost exclusively in the same species of mosquito. The habits of this mosquito do not favor exposure of many humans to its bite.

Priority was given in the laboratory to testing sentinel chicken flocks for viruses and antibodies. The appearance of western encephalitis neutralizing antibody (i. e. conversion) indicated that viral activity occurred in all five study sites in July, August, or September. The possibility of centrifugal spread from Pine Swamp, Site I, to adjacent upland areas, Sites II, III, and IV, as was suggested by 1959 hemagglutination inhibition data is still uncertain although July was the most active month in Pine Swamp, Site I, and August in the other three sites.

A surprising finding was the antibody conversions (western encephalitis) during August and September while the Culiseta melanura population indices were steadily declining from a June peak. This suggests another means of transmission, perhaps within the flocks. Intensive efforts to detect viremia in the Pine Swamp flock (Site I) were not fruitful because 1) most of the conversions, 11/13, had already occurred by August 1 when the study was undertaken, and 2) viremia probably continues no longer than one or two days. Three antibody conversions occurred during the month of August where these same chickens were being bled thrice weekly for viremia. Supporting the concept of a fleeting viremia are previous results on laboratory inoculation of chickens.

Western Encephalitis Antibody Conversion in Sentinel
Flocks, 1962 (Derived from a table prepared in August 1963)

	Before Aug 1	%	Aug.	%	Sep.	%	Oct.	%	Total Positive
Site I	11/32	34	3/19*	16	0/13	0	0/13	0	14
			(or 3)**		Or 1				
Site II	2/33	6	2/31	6-10	2/29	3.5-7	1/27	4	7
			(or 8)		or 2				
Site III	5/34	15	6/29	21-28	4/23	9.5-17	0/19	0	15
Site IV	0/33	0	3/33	9	0/30	0	0/30	0	3
			(or 2)		or 3				
Site VI	2/33	6	1/31	3-6	4/30	10-13	0/26	0	7

*Totals reduced by chickens converting or lost by intercurrent infection .

**Where earlier conversion cannot be excluded it is figured for both months.

Terminal bleedings of chickens in Site I have provided large amounts of serum, some of which show the phenomenon of inhibition hemagglutination of both western encephalitis and eastern encephalitis in the absence of neutralizing antibodies for either virus. It is hoped to determine by electrophoretic studies in what serum fraction this material resides.

REPORT FROM THE COMMUNICABLE DISEASE CENTER FIELD STATION
TAUNTON, MASSACHUSETTS

A total of 288 blood specimens was obtained from 29 species of wild birds during the 1963 spring migration period. The permanent resident bird species captured were bled, and their blood specimens were included in the survey total. Sixteen type A mist nets were utilized during 9 collection periods (from noon of one day to noon of the following day) from March 28 to May 29, 1963. The collecting was done along the dike that traverses Pine Swamp in Taunton and Raynham, Massachusetts. None of the blood specimens was positive for virus in chick embryo tissue culture systems. The past 3 years a total of 571 wild bird blood specimens was collected in the study area during the periods of spring migration, and all were tested and found negative for virus.

Small mammals were collected during the winter of 1962-63 at Pine Swamp sites I and IV in the search for latent virus from an overwintering reservoir host. At the swamp site (site I) 54 mammals were obtained and at the upland site (site IV), 47 were captured. To date, none of the organs has been tested for virus, but no virus has been isolated in chick embryo tissue culture tests completed on 52 of the blood specimens obtained.

A screened cage (3 x 3 x 7 feet), submerged in the water and mud of a pond, was found to be unsuitable for overwintering water snakes, spotted turtles, and painted turtles; whereas, 3 of 15 garter snakes and 2 spotted turtles survived in another screened cage (approximately 3 x 3 x 5 feet) in a hillside habitat from November 28, 1962 through May 20, 1963. Two of the surviving garter snakes had been inoculated with EE virus prior to overwintering and the other with WE virus. One of the EE-inoculated snakes died the day after it was removed from the overwintering cage. Blood specimens obtained May 29, 1963, from the inoculated garter snakes were positive for the respective virus with which they had been inoculated.

At that time, Aedes abserratus was the only mosquito species which could be collected in nature in sufficient quantity for vector studies. Forty of 150 A. abserratus exposed to the infected snakes the nights of May 21 and 22, 1963, fed on them. Probably because of unsuitable laboratory conditions, most of the engorged mosquitoes died the day after feeding, and all were dead by May 27.

Blood samples were taken 3 times per week during July and August from a sentinel chicken flock located at Pine Swamp site I. None of the samples collected and tested through the first week of August was positive for virus.

REPORT FROM DR. ROBERT C. WALLIS, SECTION OF MEDICAL
ENTOMOLOGY, DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC
HEALTH, YALE UNIVERSITY SCHOOL OF MEDICINE,
NEW HAVEN, CONNECTICUT

Field studies on the ecology of eastern encephalitis that have been reported from the Department of Entomology, the Connecticut Agricultural Experiment Station, New Haven, have been discontinued at that laboratory and are now part of the arthropod-borne virus program at Yale University.

A new Section of Medical Entomology in the Department of Epidemiology and Public Health, Yale University School of Medicine, has been created by Dr. Anthony M. Payne, Chairman of the Department of Epidemiology and Public Health.

The unit is designed to facilitate and expand teaching and research in the area of epidemiology dealing with insects and other arthropods of medical importance. It will provide necessary facilities for current research on problems in which insects are involved in the natural history of virus diseases. A major objective is to provide advanced study and training in this area for medical, public health, and graduate students.

With the addition of the new facility further research will be initiated that will include work on the bionomics, ecology, and physiology of mosquitoes involved in transmission of arthropod-borne virus diseases.

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

Immunologic Characterization of WEE Virus (Dr. Henderson):

The antigenic heterogeneity of some WEE viruses has been an influencing factor in the degree to which certain strains of this virus cross react in various serologic tests with antibodies stimulated by WEE strains which are not antigenically identical to the test virus. Because of these antigenic variations in WEE virus, which may be representative of many other arboviruses, authenticity, in some instances, of arbovirus identification or of data collected during the conduct of serologic surveys, for example, may be questionable. In this laboratory, therefore, studies have been designed to define some of the variations in immunologic properties associated with arboviruses, using WEE as a model.

Preliminary results of these studies have indicated the following:

- a) The antigenic properties of several plaques derived from a single WEE virus strain may be distinctly different.

b) The antigenic nature of WEE strains depends, in part, upon the quantitative distribution of distinct and different antigenic subpopulations within each strain. Of the 45 WEE strains analyzed thus far, all possess much the same subpopulation antigen varieties, and the differences between certain strains result from variation in the quantitative distribution of these distinct subpopulation antigens.

c) Using serologic markers in the form of specific immune sera prepared from antigenically different plaques derived from a single WEE strain, antigenic variations of strains are being studied, and for convenience arbitrarily classified, according to the degree of neutralization cross reactivity with the specific plaque antisera, into antigenic phases of the virus (aphasic, phase I, phase II, phase III, phase IV, etc.).

d) By passage of phasic strains in mice or tissue culture, the antigenic phase status can be changed or eliminated (aphasic). The antigenic alterations of strains from phasic to aphasic during passage in specific hosts have been termed "extraphasic conversion", and antigenic changes of strains by passage in certain hosts from aphasic to phasic have been termed "intraphasic conversion". The passage level in any one host at which the antigenicity of any one strain changes is reproducible and predictable.

Laboratory Studies on Tacaribe and Junin Viruses (Dr. Downs):

A plaque assay method has been developed for both Tacaribe and Junin viruses using primary rhesus monkey kidney cultures. Infectivity titers of both viruses range from 10^5 to 10^7 PFU/ml, depending often upon the passage level in infant mice or tissue culture and/or the degree of plaque purification. Plaques develop slowly, and accurate counts usually can be made at 7 to 10 days. The method has been useful for virus study and plaque reduction neutralization tests.

Thirty sera from military recruits residing in the southeastern U. S. (kindly supplied by Dr. J. R. Paul) were tested for Tacaribe and Oropouche neutralizing antibodies. All were negative. In addition, a group of antarctic bird sera received from Dr. Sladin were tested for Tacaribe and Junin neutralizing antibodies. All sera were negative but were positive against the Hughes virus when tested by the tissue culture neutralization test by Dr. Sonja Buckley, RFVL, New York.

Tacaribe hyperimmune mouse ascitic fluids have been prepared in large volumes using a slowly developing strain of Sarcoma 180. The average yield of immune fluid per mouse has been more than 10 ml. Complement fixing and neutralizing antibody titers in this fluid approximate titers obtained using sera from these animals.

Survey for Arbovirus Antibodies in Sera from Animals of the Connecticut Valley (Dr. Downs):

In collaboration with Mr. Leslie Williamson and his staff of the Connecticut Board of Fisheries and Game, blood-soaked filter paper discs were obtained from various birds, mammals, and reptiles of the Connecticut Valley. These were extracted and screened by the HI test against EEE, WEE, Cache Valley, Powassan, and SLE antigens. Positive reactors were obtained primarily with the group B arboviruses. Confirmatory neutralization tests are in process.

Examination of Mosquitoes Collected in Colombia for Arboviruses (Dr. Downs and Dr. Henderson):

Mosquitoes collected in Cali, Colombia, are being received through the courtesy of Dr. Sanmartin and Dr. Kokernot. Specimen pools are examined for arboviruses by inoculation of infant mice and various tissue culture systems. No isolations have been made thus far.

Installation of an Insect-proof Facility for Holding Horses or Other Large Animals (Dr. Downs and Dr. Henderson):

An insect-proof stall has been constructed for holding large animals which are to be utilized for preparing large quantities of arbovirus sero-group immune sera. This facility will provide an opportunity to inoculate animals with large doses of infectious virus for immune sera production as well as for the conduct of infectivity experiments.

Antibody Responses of Various Animals after Immunization with Plaque Purified and Unpurified Arboviruses (Dr. Saturno):

Two antigenically distinct strains of WEE virus were plaque purified. Groups of rats, guinea pigs, rabbits, chickens, and ferrets were inoculated at weekly intervals for six weeks with infectious

and formalized, purified and unpurified WEE viruses. Weekly bleedings were obtained on all animals and these sera examined against all virus antigens by the HI and neutralization tests. Preliminary results of these studies have indicated:

- a) Antibody responses of all animals were similar for both purified and unpurified preparations of each strain.
- b) The HI antibody responses (homologous) of the rat and the ferret, and the rabbit and guinea pig were similar.
- c) The rate at which HI antibody titers increased was different for chickens, rabbits, and rats (homologous series).
- d) Sera from ferrets, rabbits, and rats which were hyperimmunized with the McMillan strain, showed little cross reaction in the HI test with the Florida strain of WEE. However, sera from chickens and guinea pigs immunized with the McMillan strain gave high titers against the Florida strain.
- e) Sera from rabbits, hyperimmunized with the Florida strain of WEE did not cross react in the HI test with the McMillan strain antigen but did cross react in the plaque reduction neutralization test to titers of greater than 4.3 log PFU reduction.
- f) HI titers of sera from guinea pigs and rabbits hyperimmunized with formalized virus were high. In contrast, chickens were relatively unresponsive (HI antibody) to hyperimmunization with formalized virus.

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

At this time, we are processing survey material as it is received; the results will be tabulated later.

Surveillance in Suffolk County will include for the third consecutive year mosquitoes collected in August at or near the five sites at which infectious pools had previously been collected. In addition to attempted isolations, we are screening approximately 50 sera monthly

(May-September) from Suffolk County residents from sera submitted to our division for other tests. Neutralization tests in suckling mice include six arboviruses--EE, WE, SLE, POW, Cache Valley (Holden) and our own isolate, Flanders. All sera are also examined by hemagglutination-inhibition tests with antigens prepared from the first four of the virus strains mentioned.

Sera collected from residents of Schuyler County, a rural section in the south-central part of New York State, over a one-year period are being similarly studied.

Dairy cow sera from widely scattered areas of St. Lawrence County, in northern New York State along the St. Lawrence River, are being collected for Cache Valley antibody survey.

Surveillance in Albany County is being started this summer. Use of sentinel groups of one-day-old mice and other animals such as young rabbits are being tried. Isolation will be attempted from arthropods trapped in association with the sentinel animals.

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

No evidence of significant epidemic arbovirus activity was discovered in New Jersey during 1962. No case of overt disease attributable to arboviruses was discovered among horses, pheasants, or humans. Bird netting activities were begun during the first week of June and were continued throughout the year. Mosquitoes were trapped on a regular basis between June 5th and November 13th. Mammalian, reptile, and amphibian collections were begun in the summer and continued throughout the year.

A total of 6,809 wild birds were banded, bled, and tested during 1962. Fourteen bird bloods yielded WE. Viremic birds were found as early as June 6th--the first week of collection--and as late as December 4th. An additional isolation of WE was made from the brain and spleen of a bird found dead in a net in November. In all, WE viremia was detected in 0.22% of birds trapped in our three study areas adjoining the Atlantic shore and in 0.17% of those netted at our inland control site. Thus for the second year in a row, viremia rates in birds did not differ significantly at our control area from our shore stations. No EE was detected in birds.

A total of 32,640 mosquitoes were trapped, speciated, and tested in wet chicks in 706 pools. No arbovirus isolates were made. Similarly, no viruses have been detected in the bloods of an additional 3,153 wild birds netted between January 1 and June 30, 1963.

Table 1 summarizes results of virus isolation attempts from the brain, spleen, and blood of an assortment of Rodentia and Insectivora through June, 1963. It should be noted that WE was isolated from rodents obtained in September, November, and February, and EE was identified in rodents trapped in November and December.

Table 2 summarizes testing of the blood of an assortment of other animal species obtained in 1962. None yielded an arbovirus isolate.

Table 3 summarizes results of WE neutralization tests of rodent bloods in the wet chick. An index of 2 logs or more was considered positive. Those exhibiting 1.8 or 1.9 logs were considered doubtful. It can be seen that the incidence of WE neutralizing activity was low during the period of September 1962 through February 1963, rose very sharply in March and April, and declined very rapidly coincident with the trapping of large numbers of immature rodents born that spring. Although very few EE neutralizing studies were performed on small rodents due to limitations of sample size, one white-footed mouse trapped in April at Mays Landing showed an EE HI titer of >40 and a log neut. index of 2.9. In addition, a meadow vole obtained in June at Great Bay revealed a log neut. index of 1.8. Thus, both by virus isolation and neutralizing antibody studies, rodents appear to have been circulating EE and WE throughout the colder months of last year.

It must be emphasized that only two of the virus isolations from rodents would have been obtained had only blood been studied. Similarly of 22 mouse bloods shown to have neutralizing activity and available for HI testing, only one demonstrated significant HI activity. Had HI studies been the sole serologic technique, no significant evidence of involvement of rodents would have been found.

Since the absence of HI reactivity in the blood of wild rodents cast some doubt on the validity of our neutralizing antibody results, experimental studies were instituted using laboratory mice. These were injected by a peripheral route with small inocula of WE and have been followed, with appropriate controls, for approximately three months by serial weekly bleedings. WE HI titers, surprisingly, tend to be quite low following

documented inapparent infection (by neut. index) in some cases never achieving a titer of 1:20. More than half of the 20 mice responding with HI titers of 1:20 to 1:640 were found to have dropped below 1:20 by the 43rd day. In the overwhelming majority of mice, HI titers fell below 1:20 prior to the 66th day after inoculation. Thus, inapparent WE infection of laboratory mice led to rather poor and short-lived HI antibody responses. If this is also true of wild rodents, results reported above are easily understandable.

Although results outlined here strongly suggest that small rodents may play an important role in the enzootic cycles of EE and WE in New Jersey, it should be emphasized that other mammals may also be involved. For example, during the period October 1962 through June 1963, 24 raccoons were tested for WE neut. antibody. One was found to possess an index of 2.0 logs in wet chicks and 1.9 logs in recently weaned mice. Another yielded an index of 1.9 logs in wet chicks on two occasions. Eighteen of the twenty-four were also tested for EE neutralizing activity. Two of the raccoons demonstrated indices of 2.0 logs in both wet chicks and mice, and a third yielded 2.7 logs in chicks and 2.1 logs in mice. One fox trapped and bled in May was found to possess a WE HI titer of 1:80, a wet chick neut. index of 2.6 logs, and a recently weaned mouse index of 1.9 logs.

In conclusion, our findings to date compel us to re-examine the hypothesis that wild birds and C. melanura constitute the primary cycle of EE and WE, to study the possibility that transmission to man may occur from hosts other than birds by mediation of some vector other than C. melanura, and, finally, that some wild mammals may play an important role as overwintering reservoirs for EE and WE in New Jersey.

TABLE 1
 VIRUS ISOLATION ATTEMPTS
 BRAINS & BLOODS OF SOME RODENTS & INSECTIVORA

	Mice				Brown Rat	Shrews	Moles
	WF	Pine	House	Meadow Vole			
September	36 ⁽¹⁾	-					
October	-	-			2		
November	52 ^(2,3,4)	2 ⁽⁷⁾	1	4	2 ⁽³⁾	7	2
December	49 ⁽⁵⁾	7		2		15	2
January	20	3		6		7	1
February	33 ⁽⁶⁾	-		9		1	
March	70	-		6		3	
April	52	-		6		9	
May	72	-	2	1		9	
June	208	-	1	11		2	
Totals	642	12	4	45	4	53	5

(1) WE isolated from a pool of 3 brains of mice trapped at Great Swamp on Sept. 20th.

(2) WE isolated from brain of one captured at Great Swamp on Nov. 8th.

(3) WE isolated from spleen of one captured at Great Swamp on Nov. 20th.

(4) EE isolated from blood of a mouse captured at Great Swamp on Nov. 30th.

(5) EE isolated from brain of a mouse captured at Forked River on Dec. 27th.

(6) WE isolated from brain of mouse captured at Forked River on Feb. 7th.

(7) WE isolated from brain of pine mouse captured at Great Swamp on Nov. 25th.

(8) WE isolated from blood of a brown rat captured at Great Swamp on Nov. 2nd.

TABLE 2

MISCELLANEOUS ANIMAL COLLECTIONS, 1962
 BLOOD TESTED FOR VIRUS AND HI ANTIBODY

ANIMAL	GREAT SWAMP	GREAT BAY	MAYS LANDING	OTHER	TOTAL
Mallard Ducks				9	9
Opposum		1	1	3	5
Cat		5		5	10
Dog		1			1
Skunk	2	5			7
Raccoon	5			4*	9
Fox, Red	1	8		5	14
Fox, Grey				2	2
Muskrat	3	2			5
Woodchuck				2	2
Squirrel		1		2	3
Cottontail		3		7	10
Bullfrog		1			1
Toad		9			9
Toad, Fowler's		2			2
Toad, Common		13			13
Skink				2	2
Turtle, Box	20			2	22
Turtle, Diamond Backed	11				11
Turtle, Mud	1				1
Turtle, Eastern Painted	5				9
Turtle, Snapping	17				17
Snakes	4				4
Fish	19				19
TOTAL					187

* 1 positive WE Neut Index (2.0 logs wet chick, 1.9 logs mouse)

TABLE 3
WE NEUTRALIZING ANTIBODY IN RODENTS

	GREAT SWAMP		GREAT BAY		MAYS LANDING		FORKED RIVER		TOTAL		% POSITIVE
	# POS.	TOTAL #	#POS.	TOTAL #	#POS.	TOTAL #	#POS.	TOTAL #	#POS.	TOTAL #	
September	0	15							0	15	0
November	2	85							2	85	2
December	0	17							0	27	0
January	0(1)*	10	0	6	0	1			0(1)	17	0(6)
February			0	9	0	1	0	3	0	13	0
March	0	2	3(1)	14	1	3	5(1)	9	9(2)	28	32(39)
April	0(1)	1	7	14	7(2)	14			14(3)	29	48(59)
May	1	14	2	21	2	3	3	13	8	51	16
June	0	1	0	68	0	47	0	82	0	198	0
Total	3(2)	145	12(1)	132	10(2)	69	8(1)	117	33(45)	464	7(10)

* () Denotes number of presumptive positives.

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

Ecology of Avian Reservoirs:

During the spring and summer a regular netting program was conducted on a grid of 64 nets 300 feet apart in a tract of 132 acres of forest. Netting began in March. Nets were open in early mornings till June when the nets were opened also in the late afternoon. By August 1, 688 birds were captured belonging to 28 species. A total of 404 blood sera and 301 smears were collected.

The experiences of this year will be incorporated in another draft of our "Manual for analysis of bird reservoirs". After this revision, the manual can be sent to various field stations for criticism.

The following species of mosquitoes were collected in the area where birds were netted: Aedes triseriatus, A. fitchii, A. vexans, A. trivittatus, A. canadensis, A. intrudeus, Culex restuans, Anopheles punctipennis.

During the summer three men were trained in techniques of handling birds and obtaining blood. Also three doctoral students worked on techniques and will continue the program during the fall.

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

I. DENGUE VIRUSES

A. Typing: The testing by Miss Sather of sera prepared in several species of laboratory animals by single or multiple injections of the terminal dilution purified dengue strains is practically complete. It does not appear that these studies have contributed much to more rapid or exact typing of dengue viruses or to the successful determination of whether TH-36 and TH-Sman represent types 5 and 6 or are variant strains of the prototypes, 2 and 1, respectively. The evidence with single injection monkey antiserum tested serially up to 4-6 months post

inoculation is that there are very close relationships. However, evidence continues to accumulate that human sera demonstrate important differences in serologic response between TH-36 and dengue 2 and between TH-Sman and dengue 1. An analysis of all the available data is now being prepared.

B. Identification of Viruses from Aedes aegypti from Bangkok, 1961. One of several apparent isolates made by Dr. Rudnick, probably dengues, is being processed for identification.

C. Fluorescent Antibodies: The fluorescent antibody studies have been conducted by Dr. Jose Ordenez, a graduate student, under the direction of Dr. Atchison.

Utilizing frozen mouse brains prepared from dengue 1 infected suckling mice and the indirect CF method of FA, it was found that positive reactions occurred with mouse antisera prepared against at least five of the dengue viruses, negative or minimal reaction with Japanese B immune serum, and no reaction with Eastern equine antiserum. Apparently the specificity and sensitivity of the test is similar to that of the conventional complement fixation reaction. Exploratory studies have been made using type 3 dengue virus in very early mouse passage before titers are elevated and preparation of CF and HA antigens is possible. Results were encouraging, suggesting that this may serve as an important assist in early identification of new isolates saving months of serial passaging for adaptation. Work by Miss Sumana Suthiwart-Nareuput, also a graduate student working with Dr. Atchison and Miss Sather, revealed good CF-FA reactions with dengue virus type 1 in hamster kidney and KB cell cultures.

II. TRINIDAD VIRUS

An agent has been isolated in suckling mice from a serum specimen collected 2 days prior to onset of a febrile illness occurring 14 days following exposure of one of our university professors in bat caves in Trinidad. The agent produces illness and death in suckling mice in 3-5 days with occasional deaths occurring in 10-12 days at the endpoint dilution. The virus after 12 passages in suckling mouse brain titers $10^{-3.0} - 10^{-3.5}/0.01$ ml i.c. The sixth passage mouse brains failed to produce an active hemagglutinin. Illness and death occurred in 4/16 suckling mice inoculated with a serum collected 2 days post onset but it was not possible to establish the agent in serial passage. Mouse immune serum is being prepared against the agent which is still in serial passage.

The patient has low-titered group B HAI antibodies with no titer change. This is probably due to previous yellow fever immunization. Neutralization tests with this low titered agent have been unsatisfactory.

III. FLORIDA STUDIES:

Assistance has been given in the identification of virus isolates made at the Jacksonville, Florida, laboratory. Three tentatively identified as SLE virus were from the Tampa Bay area and one from Birmingham, Alabama. Identification of two of the agents isolated from human brain material as St. Louis virus was confirmed by cross neutralization and by vaccination-cross challenge. One agent, isolated from Anopheles crucians, proved to be a strain of Cache Valley-like virus. The agent, supposedly isolated from a human brain in Alabama, is apparently a mixture of two viruses, one component of which is herpes simplex and the second a group B virus well neutralized by SLE antisera but not SLE as shown by vaccination-cross challenge studies. A fifth virus isolated from Aedes taeniorhynchus at MacDill AFB, Florida, was sent to us by Captain Hillis and Captain Hodapp as possibly related to California virus. In our tests it is not California virus. Captain Hodapp has since shown a relationship to Tensaw virus and our studies, although incomplete, tend to confirm this.

IV. VIET NAM SERA:

An epidemic of dengue-like disease occurred in 1960 in North Viet Nam. As a result of correspondence between Dr. Sabin and Dr. Prochazka, small amounts of paired sera were sent to us from Czechoslovakia for serological tests against the dengue viruses. Paired sera from nine patients representing both Czechoslovak and Vietnamese, were tested by complement fixation using dengue antigens (sucrose-acetone extracted) prepared against the 6 types or strains following terminal dilution purification. One person had a significant increase to all dengue antigens with the highest titers against D², TH-36, and TH-Sman. One other person had a dengue response in low titer and only to TH-36 and TH-Sman. The other 7 persons by our tests were probably not infected with dengue virus. Rise in antibodies to chikungunya virus did not occur. Clinical summaries are not available at this time.

V. PHILIPPINE STUDIES:

Sera were collected by us during the summer of 1956 from febrile school children in the Philippines. Most of the children fell in the 7-9 year age group. Serum pairs, spaced approximately 3 weeks apart, were obtained from 183 children who were febrile when first bled. These have now been tested by HAI against selected group B agents, including dengue 3, JBE, MVE, TP21, and Ntaya; against selected group A agents including EEE, chikungunya, P 886 (Sindbis), and Semliki Forest; and against California and Bunyamwera viruses. So far, 11/183 have shown rises or questionable rises to the B group and the first serum specimens (acute) from these 11 are now being tested for virus. One probable virus isolation has been made from the acute serum of a group B converter. Surviving mice have resisted dengue challenge. A high percentage of the others which did not change titer have shown antibodies to group B; none has had HAI antibodies to Bunyamwera and the majority were negative for group A. Two persons with group A antibody, on the basis of quantitative serology, probably had previous chikungunya infections.

VI. ST. LOUIS VIRUS:

Studies are in progress by Dr. Nagai, a post doctoral fellow, to study plaque development by a number of St. Louis virus strains under a variety of conditions. Encouraging results have been obtained with several strains, especially the Hubbard strain and a Florida strain. Plaques are small but distinct and readily counted. The purpose of this is to develop a plaque neutralization test for human diagnostic work and for bird and human antibody survey studies. Mouse neutralization tests have always appeared to have very low sensitivity for this type of work.

VII. LIVE ATTENUATED JBE VIRUS VACCINE FROM STRAIN OCT-541:

A. The last terminal cancer volunteers to be given the 24°C line of attenuated live virus were given dosages apparently equivalent to or greater than the first two who had developed viremia and antibodies. Results were negative for viremia and for antibody response. Still larger doses will be tried next.

B. Work has begun by Captain French, a graduate student, with a 37°C line of attenuated virus. It is being cloned and assayed in animals. The infectivity of this as well as the pathogenicity for mice is greatly

reduced, but the ratio of infectivity to pathogenicity appears more favorable for use as a vaccine than that of the 24°C line.

VIII. FORMALIN INACTIVATED JBE TISSUE CULTURE VACCINE:

This work is being done by Dr. Medhat Darwish, a graduate student. Two strains of JBE virus were explored for possible use as formalin inactivated tissue culture vaccines. Both of these strains are grown in hamster kidney cells (HKTC) in 199 medium with 2% human albumin. The strains explored are:

1. OCT-541 attenuated strain.

Growth curves were studied at 30°C for both the fluid and total harvests. Titers of about $10^{8.0}$ and $10^{9.0}$ per ml can be obtained for the fluid and total harvests respectively. Half life determinations were carried out in the medium at a pH of 7.1 at both 30° and 37°C. These half lives were 14 hours and 5-1/2 hours respectively.

The virus was tested at regular time intervals in the same medium for inactivation by different concentrations of formalin at 30° and at 37° C at a pH of 7.1. The inactivation curve was linear following the first few hours, when a more rapid change occurred. The 1:4000 dilution of formalin was selected on the basis of a reasonable inactivation period and non-interference with the sensitivity of virus detection by HKTC since it was shown testing can be carried out directly without dialysis or neutralization in this concentration of formalin. Thirty degrees C was chosen in preference to 37, as it reflects a better ratio of virus inactivated by formalin versus temperature alone. Using the NIH minimum requirements for JBE vaccine potency, mouse immunity tests were performed after various periods of inactivation. The inactivated fluid harvest of titer 10^8 per ml did not fulfill the requirements when inactivation was carried out for three times the period of time required to give negative tests for live virus, while the inactivated total harvest of titer $10^{8.5}$ per ml proved satisfactory through a similar triple inactivation period. Live virus of the same lot gave slightly greater protection than the inactivated, possibly due to a low degree of infectivity. Obviously, however, formalin treatment did not cause much, if any, loss of antigenicity.

Hemagglutinin (HA) studies in 199 medium with 2% HA showed a direct correlation of the HA titer and the infectivity titer (I). The log I/H ratio is about 6.5. On inactivation with formalin, the loss in HA titer is from 2- to 4-fold, but the final HA titer is apparently directly related to antigenicity.

2. HKTC adapted Nakayama strain:

Mouse virulent Nakayama virus of titer $10^{9.5}$ per ml can be prepared in the same medium described above by harvesting the supernatant fluid only and no increase in titer was observed from using a complete harvest with cells. Half life estimation in the medium at a pH of 7.1 at 30° and at 37°C was 11 and 4 hours respectively. Inactivation of the virus in the same medium by 1:4000 formalin at a pH of 7.1 at 30° and 37°C was not linear after the first drop in titer. Thirty-seven degrees C inactivation was selected for the same reason that 30 was chosen for the OCT-541 attenuated strain. Mouse potency tests are still in progress, but from one pilot test it does not appear to be a better antigenic strain for mice than the attenuated OCT-541. Thus, the greater safety of the attenuated strain appears to offer great advantages if equal original virus titers are obtained.

Nakayama virus showed the same relation of infectivity to HA as the attenuated strain. The log I/H ratio is about 5.9. After 1:4000 formalin inactivation the loss in HA titer was also from 2- to 4-fold.

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

Immunological Studies on Arboviruses:

Studies on the immunologic overlap among arboviruses have been continued in these laboratories by Dr. W.P. Allen. Results of recent investigations on cross-protection among Group A viruses have shown that mice immunized with Semliki Forest Disease (Semliki), Gulu (O'nyong-nyong), AMM2021 or AMM2354 viruses are resistant to

challenge with 1000-5000 LD₅₀ of high passage Chikungunya virus. The latter three immunizations also elicited significant cross-protection against challenges with Semliki virus. Of the four immunizations, only Semliki virus immunization substantially protected mice against challenge with VEE (Trinidad) virus and only Gulu virus immunization elicited a protective effect against EEE virus. In all, ten different Group A viruses have been shown to protect mice against Chikungunya and Semliki viruses. The protective overlap by Group A viruses appeared to be group specific, and it did not appear to involve any interference phenomena since no cross-protection against Louping ill, Bunyamwera, or Colorado tick fever viruses was detected in mice immunized with Mayaro, Chikungunya, or attenuated VEE viruses.

Certain cross-protections also occur among the Bunyamwera group of viruses. These were revealed in preliminary experiments and are summarized in the accompanying table. Generally, cross-protection was greater following two immunizing doses of the same virus than after one dose. Greater difficulty in effectively and uniformly immunizing mice was encountered with these viruses than with Group A viruses.

TABLE OF CROSS-PROTECTION IN MICE FOLLOWING ONE OR TWO IMMUNIZING
 DOSES OF VIRUSES OF THE BUNYAMWERA GROUP

Virus	Immunization ^{a/} Doses	Cross Protection	Virus	Challenge ^{b/} Route
Bunyamwera	2	+ ^{c/}	Germiston	IP
	2	-	Cache Valley	IC
Chittoor	2	+	Bunyamwera	IP
	2	+	Germiston	IP
	2	+	Cache Valley	IC
Ilesha	1	-	Bunyamwera	IP
	2	+	Bunyamwera	IP
	2	-	Germiston	IP
	1	+	Cache Valley	IC
	2	+	Cache Valley	IC
	1	+	Chittoor	IC
	2	+	Chittoor	IC
	2	+	Kairi	IC
Cache Valley	1	+	Bunyamwera	IP
	2	+	Bunyamwera	IP
	2	-	Germiston	IP
	1	-	Chittoor	IC
	1	-	Kairi	IC
	1	-	Ilesha	IC
Kairi	2	+	Bunyamwera	IP
	1	-	Germiston	IP
	2	+	Germiston	IP
	2	-	Cache Valley	IC
	1	-	Chittoor	IC
	2	+	Chittoor	IC
	1	-	Ilesha	IC
	2	+	Ilesha	IC

- a. Mice were 10-14 gm when immunized; ca 25 gm when challenged.
- b. Challenge doses adjusted to contain approximately 100 LD₅₀ and were lethal for 80 to 100 per cent of nonimmunized mice. Mice challenged 3 to 4 weeks after last immunizing dose.
- c. + Survival of greater than 50 per cent of challenged mice
 + Survival of less than 50 per cent of challenged mice
 - No evidence of protection

Single immunizing doses of Bunyamwera or Chittoor viruses did not protect mice effectively against homologous challenges; all other immunizations were protective.

REPORT FROM UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE
BALTIMORE, MARYLAND

Enhancement of West Nile Virus Neutralization by Fresh Normal Serum:

(Dr. O. R. Eylar). The capacity of a heat-labile component of mammalian serum to enhance specific neutralization has been described for a number of viruses including dengue, Simbu, St. Louis encephalitis and western equine encephalomyelitis. This report will show that neutralization of West Nile virus, when tested in a tissue culture system, is greatly enhanced by the addition of fresh, normal human serum to the neutralization mixture.

The virus employed in this investigation was the Egypt 101 strain of West Nile virus adapted to mice by serial intracerebral passage. Seed virus was in the form of 20 per cent infected suckling mouse brain in serum-saline diluent. Hyperimmune rabbit serum was prepared by weekly intravenous injections of high titer seed virus into New Zealand white rabbits. Prior to use in neutralization tests this serum was heated at 56°C for 30 minutes.

Depending upon the final pH desired, both virus and antiserum were diluted in either borate-KCl buffer pH 8.5 or phosphate buffer pH 7.0. In either case, 5 per cent heat-inactivated rabbit serum was included in the buffer system to stabilize virus. Diluted virus, antiserum and accessory factor were temperature equilibrated separately for 10 to 30 minutes prior to interaction. After initial mixing of virus, accessory factor and/or antibody, aliquots of the reaction mixture were removed on a timed basis and immediately diluted 1/100 in iced borate-KCl buffer at pH 8.5 containing added magnesium ion. Surviving virus was assayed in chick embryo monolayer cultures by addition of 0.1 ml aliquots to the cell sheets. The details of the assay procedure have been presented in an earlier issue of the information exchange.

Preliminary neutralization tests at 37°C employed as diluent borate buffer because an increased virus stability at this pH had been established. Neutralization by hyperimmune rabbit serum at final dilutions of 1/500 and 1/1000 (and in the absence of accessory factor) plotted as log surviving virus against time, yielded a straight line curved through 90 per cent virus neutralization, thus the form of a first order reaction. The neutralization rate constant, obtained by the method originally described by Kalmonson and co-workers, was approximately $K = 23$.

Since the results of earlier survey experiments had indicated that this antiserum pool was potent, having a neutralization index of $10^{5.8}$, and because low neutralization rate constants were obtained at pH 8.5, additional studies were initiated using phosphate buffer diluent at pH 7.0. With a final serum dilution of 1/1000, neutralization at 37°C occurred more rapidly at pH 7.0 than at pH 8.5. Under these conditions, virus neutralization appeared to follow a first order reaction plot through approximately 99 per cent virus neutralization and the rate constant doubled to $K=38$.

To determine the influence of accessory factor on the neutralization kinetics similar experiments were performed utilizing phosphate buffer at pH 7.0. Neutralization tests were carried out at 37°C. Accessory factor in the form of fresh normal human serum was drawn from donors with no evidence of group B arthropod-borne virus exposure. For use in neutralization tests accessory factor was temperature equilibrated at 37°C and added to the virus-antiserum mixture at a final concentration of 25 per cent. Addition of accessory factor to the neutralization mixture increased the rate constant by approximately 3-fold. This enhancement of neutralization is seen in the reduction of time required for 90 per cent virus neutralization. In the absence of accessory factor, approximately 60 minutes were required for 90 per cent neutralization, while in its presence, 90 per cent neutralization was accomplished in 20 to 25 minutes.

That this enhancement was the result of a heat labile component of normal serum was determined by performing similar experiments utilizing accessory factor heated at 56°C for 30 minutes and by utilizing 25, 50, and 75 per cent accessory factor in the absence of hyperimmune antiserum. In the presence of heat-inactivated accessory factor, the neutralization rate returned to a level comparable to that observed when accessory factor was omitted from the reaction mixture. When 25, 50, and 75 per cent accessory factor was allowed to react with virus in the absence of neutralizing antibody, no virus neutralization was observed. Arrhenius curves were plotted and the activation energy for West Nile virus neutralization by hyperimmune rabbit serum at pH 7.0 in the absence of accessory factor was approximately 13,000 calories while under similar conditions but in the presence of accessory factor the activation energy was approximately 17,000 calories.

The results of these experiments add another virus to the growing list of arthropod-borne viruses whose neutralization is enhanced by the

action of the as yet undefined substance termed accessory factor. By the use of neutralization kinetics, it has been possible to demonstrate unequivocally the enhancement of neutralization by this substance.

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

During the period from 1 January 1962 to 1 May 1963, 145 doses of western equine encephalitis virus vaccine were distributed by the Division of Veterinary Medicine, Walter Reed Army Institute of Research, to various laboratories throughout the U. S. , for prophylactic immunization of high risk personnel. Paired samples of pre and post immunization sera were tested for the presence of serum neutralizing (SN) antibodies to western equine encephalitis.

The vaccine used was a purified chicken embryo preparation produced and lyophilized in 1950. The seed virus was the B-11 strain of western equine encephalitis. Three doses of 0.5 ml were given subcutaneously on day 0, 7, and 21. Post-immunization sera were collected on day 35.

The paired sera were tested against varying dilutions of the homologous B-11 strain of western equine encephalitis virus. Equal amounts of serum and virus were incubated at 37°C for one hour, and 0.03 ml of this serum-virus mixture was inoculated IC into each of 5 mice. The mice were observed for 14 days. Protection against 1.2 or more logs of virus was considered a positive response.

The following table summarizes our results for this western equine encephalitis immunization study.

Serological Survey of Individuals Immunized with Western
Equine Encephalitis Vaccine - (Strain B-11)

Source of Paired Sera	No. of Ind. Vaccinated	No. of Ind. Positive	No. of Ind Negative
Ft. Dodge Lab., Iowa	47	9	38
N. Y. State Health Dept.	9	2	7
Ft. Detrick, Maryland	25	1	24
Univ. of Miami, Florida	12	4	8
Univ. of Arizona	13	0	13
Bandy Lab., Texas	12	5	7
Univ. of Wisconsin	27	11	16
TOTAL	145	32	113

REPORT FROM DRS. M. W. PROVOST AND R. P. DOW
FLORIDA STATE BOARD OF HEALTH ENTOMOLOGICAL RESEARCH CENTER
VERO BEACH, FLORIDA

It has been established that Culex nigripalpus, vector of SLE, exhibits a remarkable cycle of flight activity correlated with rainfall on almost a day-to-day basis. This was established from three sampling methods: light trap, bait trap, and truck or "air-sampling" trap. This has important bearings on adult control and on virus transmission. The prolonged inactivity during rainless periods may permit the multiplication of the virus in the infected mosquito and prolong the mosquito's life--prerequisites for transmission which the usual survival curves for mosquitoes can scarcely permit.

Parakeets (budgerigars) have been found more effective than 2-to 4-week-old chicks in portable bait traps used to collect C. nigripalpus. In 54 paired or otherwise balanced trap settings, parakeets attracted an average of 18.2 C. nigripalpus and chicks an average of 14.5. The difference seems consistent but has not been shown to be significant. In a test that exposed the birds to mosquito attack for 2-hour periods, the proportions of C. nigripalpus that obtained blood (any amount) were 4 per cent in the parakeet-baited traps and 18 per cent in the traps baited with chicks (cockerels).

In a preliminary test of the relative suitability of cockerels and pullets as sentinel chicks, 223 C. nigripalpus were trapped by the cockerels and 183 by the pullets. During the all-night exposure period, 78 per cent of the C. nigripalpus obtained some blood in the cockerel-baited traps, and 96 per cent in the traps baited with pullets.

REPORT FROM TAMPA BAY REGIONAL ENCEPHALITIS LABORATORY
FLORIDA STATE BOARD OF HEALTH, TAMPA, FLORIDA

The Tampa Bay Regional Encephalitis Laboratory (TABREL) has found very little evidence of St. Louis encephalitis or other arbovirus activity in the Tampa Bay area of Florida during the spring and early summer of 1963.

Human diagnostic specimens have been received on 201 suspected viral infections of the central nervous system during the period January 1

through 26 August. In seven individuals there have been low or constant levels of HAI antibodies against SLE indicating past infection with a Group B agent. In one case, a changing titer of $<1:10$ to $1:40$, and return to $<1:10$ within a 52-day period, suggested the possibility of recent infection with St. Louis encephalitis. There have been no serologic responses to Western equine HA antigens; one individual had a constant level of $1:20$ HAI antibody against eastern equine in paired sera, without concomitant clinical disease.

Serological surveys for inapparent human infection have now been completed in representative areas of the 4 counties around Tampa Bay (Table I). These have all been probability samples of their respective areas, permitting generalizations to the total population in each area to be made with assurance. Of the total of 3081 persons bled and tested, 167 or 5.4% had HAI antibodies against SLE at a level of $1:20$ or greater. In one area (Clearwater), examination for SLE-CF antibodies has been completed. These were present in 4.2% of the survey population. The small group of 89 individuals bled in St. Petersburg in 1963 was a portion of 330 individuals bled in January 1960 whose early sera were available for comparison testing. Of 73 individuals who had an HAI titer of $<1:10$ against SLE in 1960, 7 converted to a titer of $1:20$ or greater by 1963. There were 16 with titers of $1:10$ or greater in 1960 and 10 of these reverted to $<1:10$ by 1963. Extensive serological analyses on these survey bloods and on patients from the 1962 epidemic who were serially bled for periods up to one year, are in process in CDC and in the University of Pittsburgh arbovirus laboratories.

Collection of adult female mosquitoes in modified chicken baited traps has been carried out bi-weekly in 28 standard sites. The results of these collections and their examination for viral agents are summarized in Table II. Although similar collection data from previous years are not available for comparison, the low numbers of Culex nigripalpus collected this spring have been noteworthy.

Over 1200 pools of these mosquitoes have been inoculated into suckling mice. In eight of these viral isolations have been obtained, all from one species, Aedes infirmatus. These agents are in various stages of identification. To date a number of similarities shared by these isolates have been observed. The incubation period for the primary isolation attempt has been from 7-8 days. Upon passage, this is reduced to 60-64 hours for a 10^{-3} dilution.

Serological studies have been initiated on most of the isolates. Crude brain suspensions of SM3 material have failed to yield a demonstrable hemagglutinin. In addition, the crude antigens when utilized in the CF tests against EE, WE, or SLE hyperimmune sera have failed to fix complement. Serum neutralization tests employing SLE or Tensaw antisera, failed to confer significant amount of protection.

Pathogenicity for three- to four-week-old mice by the i. c. route has been variable. Material from the 3rd mouse passage of the first five isolates did not kill adult mice in the range of 10^{-1} to 10^{-9} dilutions. Isolates obtained later in the month of June have been pathogenic by the i. c. route with an LD₅₀ between 10^{-5} and 10^{-6} . These isolates are presently undergoing further identification studies at the University of Pittsburgh and the CDC virology laboratories.

Sentinel chicken flocks were established in late March in 25 representative locations in 3 counties. Through August 23, groups of 100-125 chickens have been exposed during each of eight successive 3-week periods. Of the approximate 1000 birds involved, 10 have shown conversion from no antibody to low levels of 1:10 or 1:20 HAI antibodies against SLE. Each of the 3 counties were represented in the 7 exposure sites from which these 10 converting birds were obtained.

Concurrent with this program, approximately 600 nestling or fledgling wild birds have been bled for HAI antibodies. There have been low titers (1:10 or 1:20) against EE in 14 birds and against SLE in 8. These equivocal SLE titers have been obtained from doves, yellow-crowned night heron, brown pelican, American egret, and redwing black birds; the EE antibodies were found in doves, a stilt, skimmers, and a Wilson's plover.

The significance of these low HAI antibody titers will not be known until confirmatory studies with SN antibodies are completed. In the meantime, the large number of negative serological findings in sentinel chickens and nestling wild birds, combined with the lack of evidence of human or arthropod infections with SLE, reinforces the impression that there has been very little arbovirus activity in the first six months of 1963 in the Tampa Bay area.

TABLE I

The Results of Serological Surveys for HAI Antibodies Against SLE
in Residents of the Tampa Bay Area, 1962-63

Area	Number Examined	Number Positive*	Per Cent Positive
Clearwater (Pinellas County)	1039	83	8.1
St. Petersburg (Pinellas County)	89	12	13.4
Tampa (Hillsborough County)	510	29	5.7
Rural (Hillsborough County)	531	12	2.2
Bradenton-Sarasota	<u>912</u>	<u>31</u>	<u>3.4</u>
Total	3081	167	5.4

Positive- HAI Titer of 1:20 or greater

TABLE II

Adult Female Mosquito Collections in 2⁸ Bi-weekly, Chicken Baited Traps:
 Hillsborough, Pinellas, Manatee and Sarasota Counties
 Period January-July, 1963.

Month	Total Mosq. Collection	<u>C. nigripalpus</u>		<u>Aedes infirmatus</u>	
		No. Coll.	Pos.Pools	No.Coll.	Pos.Pools*
Jan.	2116	560	0	3	0
Feb.	4237	660	0	10	0
March	5367	420	0	1597	5
April	1513	138	0	384	1
May	1307	166	0	67	0
June	3222	1658	0	214	1
July	5101	3502	0	179	1

* Positive pools for Aedes infirmatus are unidentified.

Preliminary tests indicate they are not SLE, WE, EE, or Tensaw.

REPORT FROM ANNIE R. BEASLEY AND MICHAEL SIGEL
UNIVERSITY OF MIAMI SCHOOL OF MEDICINE
CORAL GABLES, FLORIDA

Hamster kidney cell tissue cultures obtained from Dr. M.G.P. Stoker as an established cell strain, designated BHK21, are proving highly useful in our studies with dengue virus. Following inoculation with dengue mouse brain preparations or virus derived from dengue carrier cultures, distinct cytopathogenic effects can readily be observed and significant changes (3-4+ CPE) may occur within four days. Assays in this tissue culture system give titers approximating those obtained in the newborn mouse host. Precise comparisons of infectivity endpoints in mice and BHK cells are currently under study.

Factors controlling resistance of dengue carrier cells in tissue culture to superinfection with other viruses:

We have previously shown that KB cells in tissue culture could become permanent carriers of dengue virus. This system has been characterized by survival and growth of the cells as well as continuous propagation of the virus. These carrier cells were found to differ from normal KB cells in several parameters, including resistance to superinfection. In previous experiments, it was demonstrated that establishment and persistence of the carrier state and the development of resistance to superinfection were associated with a) initial partial resistance of the cells to dengue virus, b) alteration in the virulence of the virus, and c) the production of a substance resembling, in many respects, interferon. More recently, it was observed that the status of resistance may, in addition, be influenced by nutritional factors.

The carrier cells cultivated and maintained in Eagle's basal medium (BME) supplemented by serum have manifested resistance to both polio and EMC viruses, with the latter virus serving as an especially good indicator of resistance and interference. When Eagle's minimal essential medium (MEM) was substituted for BME, there was a marked decrease in the resistance of the cells to challenge by EMC virus. The effect appeared to be temporary inasmuch as cells returned to BME from MEM regained their full resistance to superinfection. It has not yet been determined whether this environmental (nutritional) effect occurs by way of action on the cell (altered cell growth or altered production of interferon-like substance) or by way of some indirect factors.

REPORT FROM DR. D. D. STAMM
VIRUS ECOLOGY LABORATORY, ARBOVIRUS UNIT
COMMUNICABLE DISEASE CENTER, ATLANTA, GEORGIA

Field studies in Baldwin County, Alabama, 1957 - 1963

Studies on arbovirus activity in wild bird populations have been in progress 14 miles northeast of Mobile, Alabama, since 1957. In 1957 and 1958, isolation tests were performed by intracerebral inoculation of 3-week-old mice. From 1959 to date, isolation tests were performed either by intracerebral inoculation of suckling mice or subcutaneous inoculation of 1/2-day-old chicks.

The results of virus isolation tests on more than 9000 bird blood samples are shown in Table 1.

Some striking trends in the presence of EE and WE virus are apparent. From 4280 samples collected from January through June, only one isolation of EE virus was made. From 5036 samples collected from July through December, 74 isolations of EE and 33 of WE virus were made. With the exception of July, 1963, WE virus appeared later and was isolated from fewer samples than EE virus. WE virus may have been absent from the study area during 3 of the seven years during which studies were conducted.

In this area of Alabama, the northward flow of migrating birds begins in March, is heaviest in April, and has ended by June. Birds which breed farther north in the U.S. and Canada begin passing through the area on their way south to Central and South America about July 10. Therefore, with the exception of the one isolation in June, EE and WE virus activity in this Alabama area was detected after there was opportunity for viruses to be re-introduced into the area from farther north by migrating birds. These observations are consistent with the hypothesis of reservoirs of these viruses in northern U.S. or Canada set forth by Dr. Harald N. Johnson. Recent findings in New Jersey suggest that arboviruses are present there in a small mammal reservoir during winter months. (Personal communication, Dr. Martin Goldfield, 1963. See report of State of New Jersey Dept. of Health in this issue.)

TABLE 1

VIRUS ISOLATIONS FROM WILD BIRDS IN BALDWIN COUNTY, ALABAMA

Year	No. tested & virus isolations	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Totals
1957	Tested			27		33	39	26	131	106	40	51	113	566
	EE			0		0	0	1	2	0	0	1	0	4
	WE			0		0	0	0	0	0	2	1	0	3
1958	Tested	252	173	40	83	64	145							757
	EE	0	0	0	0	0	1							1
	WE	0	0	0	0	0	0							0
1959	Tested											172	179	351
	EE											2	2	4
	WE											0*	0	0*
1960	Tested	306	534			588		498		504	148	176	115	2869
	EE	0	0			0		0		26	9	2	0	37
	WE	0	0			0		0	*	15	6	3	0	24
1961	Tested									204				204
	EE									2				2
	WE									0				0
1962	Tested					734	69	366	306	654	407	136	310	2982
	EE					0	0	2	6	2	9	1	2	22
	WE					0	0	0	0	0	0	0	0	0
1963	Tested	147	582		464			394						1587
	EE	0	0		0			6						6
	WE	0	0		0			6						6
Totals		705	1289	67	547	1419	253	1284	437	1468	595	535	717	9316
		0	0	0	0	0	1	9	8	30	18	6	4	76
		0	0	0	0	0	0	6	0	15	8	4	0	33

*EE virus was isolated from passerines in Nov. 1959 and Aug. 1960

REPORT FROM DR. S. R. PATTYN, BACTERIOLOGY DEPARTMENT,
INSTITUTE OF TROPICAL MEDICINE PRINCE LEOPOLD
ANTWERP, BELGIUM

We studied the production of HA antigen comparatively in HeLa cells, CETC and newborn rat brain. HA antigens in TC fluids were determined after infection of monolayers at 37°C. in a serum free maintenance medium composed of Hanks BSS with 0.5% LH 80; bovine albumin (fraction V, 10%)², Tris 0.05M²⁰. HA was determined in the MM after addition of 1/5 borate saline without further extraction. Harvests were done at days 1-4 for group A viruses, days 5 and 7 for the others. The HA techniques described by Clarke and Casals with goose erythrocytes were applied.

Results:	<u>Rat brain</u>	<u>HeLa cells</u>	<u>CETC</u>
WEE	1024	0	512
Sindbis	128	2	256
Chick	128	0	0
SF	256	8	64
YF	128	32	0
WN	512	8	0
RSSE	64	16	32
Dengue 2	256	0	0
Jap B	256	4	0
Bunyamwera	0	0	0

It thus appears that CETC may be a useful medium for obtaining HA antigens for group A viruses, whereas group B viruses produce some HA antigen in HeLa cells.

HA antigens for group A viruses appear within 24 hours. The effect of serum was studied in the system SF and WEE in CETC by comparing titers obtained after addition of 5% calf serum to the maintenance medium. Results were as follows:

WEE:	MM	:512
:	MM+5% calf serum	:512
SF :	MM	:64
:	MM+5% calf serum	:4

Normal serum may inhibit HA production by some viruses in TC.

A study was made of the virus multiplication and CPE appearance of SF virus in CETC and HeLa cells at different bicarbonate concentrations: low:0.03%, medium:0.07% and high:0.22%. In HeLa cells, virus multiplication is 1 log lower in the presence of high bic. conc., as where in CETC the bicarbonate effect is not evident.

CPE is also much less pronounced in high bic. conc. than in low bic. conc. were:

high	$<10^{-2}$
medium	10^{-4}
low	10^{-5}
none	10^{-5}

The titer of CPE titration on CETC was not influenced by bic. conc.

Experiments in which the medium over HeLa cells was changed 2 hours after infection with SF virus, showed that virus adsorption on the cells was not diminished by high bic. conc. Virus synthesis was enhanced by low bic. conc. and in one instance we observed a dissociation between CPE and virus production.

Infection in medium at	After 2 hours change to	CPE	Virus titer determined on CETC
low bic.	none (control)	++	10^5
low bic.	low bic.	++	10^5
low bic.	high bic.	+(50% of tubes)	10^1
high bic.	none (control)	0	10^1
high bic.	high bic.	0	$<10^1$
high bic.	low bic.	0	10^4

That this effect of bicarbonate is due to the ion and not to the accompanying pH, was shown by an experiment in which high and low pH were obtained in the absence and the presence of high or low bic. conc.

At low bic. conc. --and high or low pH values--CPE attains constantly significantly higher titers. At high bic. conc. --and high or low pH of the medium--CPE is not observed.

The stability of SF virus incubated at 37°C. in a cell free medium and in the presence of the various bicarbonate concentrations showed a marked difference as shows the following table, giving the % survival (titrations in CETC).

	<u>0.03% bic.</u>	<u>0.07% bic.</u>	<u>0.22% bic.</u>
0	100	100	100
60'	90	90	70
120'	90	90	20
240'	33	20	2

During plaque titrations with arboviruses following the techniques of Porterfield, it was found useful to omit all serum from the overlay medium and to incorporate 10% CEE in it. Normal calf serum inhibits in various degrees plaque formation by several viruses. Addition of protamin sulfate in the overlay medium could not neutralize the inhibiting effect of the calf serum. Light also can inhibit plaque formation up to 10 or 20%.

Our studies on the effect of virus infections on pregnant mice were negative with SF, WN, Jap B viruses.

REPORT FROM DRS. A.A. SMORODINTSEV AND V.I. ILYENKO
VIRUS INSTITUTE OF EXPERIMENTAL MEDICINE
ACADEMY OF MEDICAL SCIENCES, USSR, LENINGRAD

In this section in the last two years, the following questions were studied:

I. Discovery of new foci of transmissible infections in the country as a whole. Serological examinations were first used to localize foci of transmissible infections. Attempts followed to isolate suspected agents from sick human patients and migratory birds.

A. Results of serological examinations. Sera from healthy human beings were tested for antibodies to viruses of different serological groups (A, B, and C, after Casals) using hemagglutination inhibition test.

Casals' method was used in preparation of antigens. Hemagglutination inhibition test was carried out with 1.5% bentonite absorption and 0.3% goose erythrocytes. Examination of 3638 human sera established existence of several active foci in different districts of the country. (See Table 1). Districts were identified where antibodies to antigens of the tickborne virus encephalitis group were most frequent. Other districts were found where antibodies to antigens of mosquito-borne encephalitis existed. Also, there were districts in which the sera of the population reacted to a wide spectrum of antigens in the HA test. In the examined serum samples, antibody titers were from 1:10 to 1:320, rarely 640.

B. Results of virological examinations. In newborn mice, 50 strains of virus were isolated. Sources of isolation are presented in Tables 2, 3, and 4. On the basis of previous data, the isolated viruses could be separated into three groups. One of them to group A (after Casals), second to group B, and the third one, the agents which are heterogenous and not yet identified.

C. The role of migratory birds was studied in transmission of the infectious agents in two ways.

1) HI test for antibodies in the blood of birds.

2) Virus isolations from blood and organs from the trapped birds (Table 5). From the 132 bird organs which were caught in fall, 6 virus strains were isolated. From 120 bird organs caught in spring, 2 strains were isolated. An additional 251 birds are under study at present.

II. Specific prophylaxis in tickborne encephalitis.

A. The technique was developed and we received experimental series of killed tissue culture vaccine, prepared on fibroblasts of chick embryos. The vaccine did not cause local or general reaction in adults or children but stimulated humoral immunity in more than 80% of vaccinated individuals.

B. Studies have been completed for the use of Malayan virus Langat TP-21 in live vaccine form against tickborne encephalitis. For this purpose, Langat TP-21 virus was received in 1959 from J. Casals' laboratory and cleaned by the dilution method.

Virus TP-21 multiplies rapidly in the brain of adult mice, reaching $7.0-8.0 \lg^{10}(10^7-10^8)/0.03$ ml; it is harmless when injected i. p. or s. c. Virus injected into the brain of *Macacus rhesus* in 2.10^{7-8} Ld₅₀ concentration does not affect them.

Careful observation of 608 human individuals injected s. c. with Malayan virus showed it to be harmless. In 88% of persons injected twice, antibodies were formed and neutralized Malayan virus and virus of tickborne encephalitis. Antibody titers were maintained for 6 months. At present, we are preparing a larger scale epidemiological experiment to establish the effectiveness of live vaccine prepared with Malayan virus.

III. Studies of fundamental principles in transovarian transmission of tickborne encephalitis virus. The existence of transovarian transmission of the tickborne encephalitis in *Ixodes ricinus* and *Ixodes persulcatus* was proven in the following additional experiments:

- 1) In regular transmission of the virus through the eggs in experimental infection of the ticks.
- 2) Systematic isolation of the tickborne encephalitis virus from larvae developed from ticks engorged in nature.
- 3) Possible formation of immunity in white mice injected with suspension of hungry larvae developed from ticks collected in the field.

Transovarian transmission of the virus is not evenly divided between the ova of infected female. Even in mass infection of the ticks in the laboratory, not all ova were infected, which affects the isolation of the virus from a small number of larvae. It is possible to get several generations from virus infected ticks. We examined the process of generation in all stages of development of the ticks and found the virus in all, but the regularity of virus isolation gradually diminishes. After the first generation, one or two additional passages are needed because the number of non-infected larvae increases. (Table 6) Unequal distribution of the tickborne virus in the progeny of the infected female is illustrated in Table 7, expressed as per cent values virus carrier in transovarian transmission of the virus. Experimentally, infection of larvae may reach 34-50% whereas in natural infections of ticks, it is substantially lower, usually between 3-5 to 25%.

Table #1
Results of H. I. Test on Sera of the Population from Different Foci

Foci of Infection		% of Positive H.I. with Group Antigens										
Republics	Districts	A									C	
		WEE	JBE	KFD	TP21	POW	J.E.	W.N.	S.L.	Dengue	Mari-tuba	Bunyamwera Germiston
Azerbedjan	Massalinsky	0	0	33.3	25.8	3.8	3.5	0	-	0	0	0
	Astarinsky	14.5	0	9.7	10.1	3.0	6.0	0	29.7	2.5	-	1.5
	Lenkoraysky	29.0	0	29.0	11.0	5.8	5.4	12.0	63.0	1.9	-	0
Kirgiziya	Kalininsky	3.1	13.2	1.0	3.1	6.2	5.1	0	-	0	12.5	6.2
	Iruzinsky	29.0	2.0	0	2.0	9.0	12.0	11.0	-	0	-	2.0
	Kizin-Kiya	8.0	32.0	26.0	24.0	12.0	19.0	-	-	5.0	-	4.0
Tadjekistan	Fayzabadsky	7.0	4.3	2.0	10.0	0	6.5	1.2	-	0	-	0
	Cissarsky	3.2	5.9	3.4	2.8	1.0	5.3	12.3	-	0.7	-	1.4
	Kaskozabarsky	19.0	7.0	5.0	10.6	0	25.0	15.0	-	1.4	-	2.0
Udsmurtiya	Yakmurbodya	23.0	35.0	32.0	31.0	16.0	38.0	4.0	-	4.0	-	-

Table #2

SUMMARIZED DATA ON VIRUS ISOLATION SOURCE IN FOCI OF TRANSMISSIBLE INFECTIONS

<u>Isolation Source</u>	<u>No. of Strains</u>
Human blood	1
Rodens blood	1
Bird blood and organs	8
Mosquitoes	13
Ticks	<u>27</u>
Total	50

Table #3

RESULTS OF VIROLOGICAL EXAMINATIONS ON MOSQUITOES

Mosquito Collection Places	Type of mosquitoes	No. of examined cases	No. of mosquitoes	Isolated virus strains
Udmurtiya	Aedes contans		2321	10
Azerbedjan	Aedes caspius	4	60	0
	Culex pipiens	4	130	2
	C. tritaeniorhynch	1	3	1
	Anoph. maculopennis	1	5	0
Total			2599	13

Table #4
Results of Virological Examination of Ticks

Collection Place	Type of Ticks	Development Stage	No. of Observed Cases	No. of Observed Ticks	Isolated Virus Strains
Azerbedjan	Hyaloma detritum	IM	9	607	1
	Hyaloma detritum	L	4	1100	1
	Ixodes ricinus	IM	2	26	2
	Hyal. dromedarii	IM	1	11	1
	R. turanicus	IM	5	419	4
	R. sanguineus	IM	3	307	2
	H. plumbeum	IM	2	14	0
	H. concinna	IM	1	2	0
Kazachstan	H. anatolicum	IM	7	68	3
	Arc persicus	IM	5	50	1
Tardzikistan	H. anatolicum	IM	22	770	6
Udmurtiya	Ixodes ricinus	IM	31	31	6
Total			92	2405	27

Table 5

HI Test on Avian Sera. Number of Serum Samples Containing Antigens.

Species of birds	No. of obser- vations	WEE	Sind- bis	TBE	TP	KFD	JE	WN	SL	Dengue
Finches	116	3		1			2	1		
Brambling	66						1	2		
Tomtits; blue titmice	92	2					3			
Woodpeckers	13									
Thrushes	18						2	2		
Robin	51						2	6		
Siskins	20									
Wheatears	9		1				2			
Swallows	44									
Starlings	25									
Chiff-chaffs	18									
Cuckoos	3									
Crossbills	3									
Flycatchers	5									
Sparrow hawk	1									
Woodcock	1									
Duck	40						2			
Snipe	6									
Pochard	8									
Teal	2									
Greylag goose	3									
Brent goose	3									
Coot	9					2	2			
Swan	1									
Grebes	1									
White heron	20			1		1				
Cormorant	16	1					1			
Night heron	9									
Glossy ibis	8									
Black tern	34									
Totals	725	6	1	2	0	3	17	11	0	0

Table 6

TRANSOVARIAN TRANSMISSION OF THE TICKBORNE
ENCEPHALITIS BY IXODES RICINUS

<u>Generation of the tick</u>	<u>Isolation of Virus</u>
1	*2/2**
2	5/5
3	2/6
4	1/10

**No. of parts of larvae.

*No. of parts from which virus was isolated.

Table #7

DETERMINATION OF THE PERCENTAGE OF VIRUS CARRIERS BY TRANSOVARIAN TRANSMISSION
OF THE VIRUS OF TICKBORNE ENCEPHALITIS

No. of infected ♀	No. of larvae taken from infected ♀ for feeding	No. of fed larvae	No. of nymph develop.	No. of fed nymph for observation	Amount of imago devel. from nymphs		Amount of ♀ examined for virus carrier cap.	From them virus was isolated in:	% of infected larvae
					♀	♂			
1	2386	2006	1520	500	215	243	43	20	46
2	2541	2144	1684	496	198	203	35	12	34
3	1028	983	704	605	301	208	40	21	52
Total	5955	5133	3908	1601	714	654	118	53	45

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

The Tahyna Virus:

The experiments were continued in which the multiplication of virus Tahyna--the neuroadapted and extraneural variants of strain 236--were compared in various types of cell cultures (Dr. L. Sefcovicova). There is no evidence of qualitative differences in susceptibility of primary cultures of chick and duck embryo cells and of that of hamster and rabbit kidney cells. In all investigated types of cells the extraneural variant of virus in contrast to the neuroadapted one multiplied in the first passage without or with delayed cytopathic effect. In the medium of individual cell cultures, virus was demonstrated by i. c. titrations on young mice in maximal titres of $10^{2.5} - 10^{5.5}$ LD₅₀/0.1 ml. The neuroadapted variant showed values of $10^4 - 10^6$ i. c. LD₅₀/0.1 ml and $10^4 - 10^{5.5}$ CPD₅₀/0.1 ml, respectively.

The relationship between the size of inoculum and viremia and the immune response in experimentally infected potential reservoir animals was investigated (Dr. A. Simkova). Inapparent infection accompanied for 3-4 days with viremia developed in young rabbits, hares, and hamsters after inoculation with different amounts of the extraneural strain "236" of Tahyna virus. The minimal dose of virus necessary to cause viremia in rabbits or hares weighing 400 g. was about 0.1 - 0.2 LD₅₀ of virus per g. body weight, while about 2.0 LD₅₀/g. body weight were necessary to cause viremia in 80 g. hamsters. The maximal level of virus in the blood from these animals, $10^{2.2} - 10^{3.5}$ i. c. LD₅₀ per 0.03 ml blood, was not affected by the amount of virus inoculated. Virus neutralizing antibodies appeared in sera from young rabbits, hares, and hamsters immediately after viremia.

These quantitative studies suggest that these young mammals can be regarded as short-term reservoir animals of Tahyna virus in nature.

The role of domestic animals in the ecology of the Tahyna virus was followed in further experimental studies (Drs. Bardos and Jakubik).

A one-year-old horse was inoculated subcutaneously with 3.42 log of extraneural variant of the Tahyna virus, strain "236". Viremia lasting

from the second to the fifth day was found only by i. c. inoculation of suckling white mice. Virus neutralizing antibodies were present on the 7th day.

Five suckling pigs of 1.8-2.0 kg weight were subcutaneously inoculated with 3.2 to 3.4 log of the same virus. Viremia was found from the second to the fifth day. The titer of the virus in the blood of the suckling pigs reached level of 2.5 log on the third day in an i. c. titration in young mice (7.0 g). Virus neutralizing antibodies were present on the 10th day.

Two calves (2 and 5 days old) were inoculated subcutaneously with 3.36 log of the same virus. Viremia was present from the second to the fifth day but only by intracerebral inoculation of suckling white mice. Virus neutralization antibodies were present on the 10th day.

The experimental pathogenesis of Tahyna infection in white mice was further studied (Dr. Bardos).

In subcutaneously or intracerebrally inoculated white mice from 4.9 to 9.0 g of body weight, we found on the 4th and 5th day a higher level of virus in the suspensions from the exsanguinated lungs than in the blood. These results were achieved in 9 out of 11 experiments.

In the same experiments, we also found on the 4th and 5th day a higher level of virus in suspensions of striated muscles. This was the case in 6 experiments out of 11.

In specimens of spleen, kidney, liver and heart muscle, higher levels of virus than in the blood were only occasionally found.

After intracerebral inoculation of virus, the titers of virus in the brains of moribund mice reached a level of 6.0 log.

The production of interferon was not found in the organs of subcutaneously inoculated mice. We found interferon only in the brains of moribund mice after their intracerebral inoculation. One hundred to 1000 TCID₅₀ of the EMC virus was inhibited in monolayers of L cells to a titer of 1:2 to 1:8.

The Calovo virus:

The role of domestic animals in the circulation of the Calovo virus was experimentally studied (Drs. Bardos and Jakubik).

Five suckling pigs of 1.3 - 1.5 kg of body weight were inoculated s.c. with 3.42 - 3.62 log of a neuroadapted strain ("184"). We found a viremia lasting 3 to 5 days. The level of the viremia reached a titer of 1.6 log titrated i.c. in young white mice. Virus neutralization antibodies were present on the 10th, 14th, and 28th days.

Three one to two year old horses were inoculated subcutaneously with 3.20 - 3.30 log of the same virus. The viremia lasted from day two to day five. The titer of the virus reached on the third day a level of 1.0 log i.c. in white mice.

Two calves were subcutaneously inoculated with 3.70 log of the same virus. We found a viremia lasting from the second to the fifth day by i.c. inoculation of suckling white mice.

Serological survey by HIT:

The survey was undertaken by means of using blood samples from the healthy population and from the hospital patients living in the southern regions of Czechoslovakia, known as regions of mass occurrence of mosquitoes (Dr. Cupkova).

In 364 sera tested, 5.5% group A antibodies with a titer of 1:40 and more against 4-8 units of antigen were detected. EEE, WEE, Semliki forest, and Sindbis viruses were used in the test. The sera were treated with bentonite and kaolin and acetone. No virus neutralizing antibodies were found against the WEE or Semliki forest viruses in these sera.

HI antibodies against viruses of the B group (dengue 1, West Nile, St. Louis) were present in a titer 1:40 and more in 34.6% of sera out of 317 tested. No virus neutralizing antibodies were found against these viruses.

In 571 sera tested, there was an incidence of 16.1% HI antibodies against Tahyna virus antigen in a titer of 1:40 or more. There was a 10.7% incidence of Calovo positive HI reactions in 485 sera. Four to eight units of antigen was used in all tests.

REPORT FROM DR. H. MORITSCH
INSTITUTE OF HYGIENE, UNIVERSITY OF VIENNA, AUSTRIA

A survey on the presence of antibodies to tickborne encephalitis (TBE) virus in forest workers was carried out in 1962/63. The main aim of the study was to determine whether infections with TBE virus are especially frequent in these persons who are exposed to tick bites while working to establish whether TBE must be listed among the occupational diseases. Forests in Lower Austria, Styria, and in the Burgenland were chosen for this purpose.

A first blood sample was taken from 1255 donors in February 1962 and tested in the tissue culture (HeLa cells) neutralization test (NT). Nineteen per cent of the sera exhibited neutralizing antibodies. The percentage of positive sera increased with age and varied considerably according to the area of employment. For example, a low incidence of antibodies (3%) was found in people working in forests situated along the Danube, whereas 41% of 230 persons employed in 3 different areas where TBE was known to be endemic were positive in the NT. For comparison, sera of 1412 normal residents living in the endemic district of Neunkirchen were tested also. Positive results were obtained in only 14%. Statistical evaluation of these figures, which were standardized on the Austrian population, showed that the difference between the residents of the Neunkirchen area and the forest workers of the endemic districts is very significant.

In order to establish the rate of antibody conversion during one tick season among those forest workers who were negative in the first blood sample, 726 persons were bled again one year later. It could be demonstrated that 4 people (0.55%) had acquired neutralizing antibodies during 1962. One of these workers had been hospitalized in 1962 with TBE, whereas the other three persons had apparently not been ill. It is of interest that CF antibodies were detectable only in the serum of the person with TBE in the history.

The higher risk of infection with TBE virus for forest workers as compared with the normal population is also indicated by the comparison of their morbidity rates. In 1962, out of 87,987 persons living in the district of Neunkirchen, 16 were hospitalized with TBE (morbidity rate: 18 per 100,000). In contrast, among 7,141 forest workers living in Lower Austria, 7 cases of TBE had occurred during the same period (morbidity: 98 per 100,000). This difference is statistically also very significant.

REPORT FROM DR. J. VESENJAK-HIRJAN,
SCHOOL OF PUBLIC HEALTH "ANDRIJA STAMPAR",
MEDICAL FACULTY, ZAGREB, AND
DR. M. JUNG, NATIONAL INSTITUTE OF HEALTH,
LJUBLJANA, YUGOSLAVIA

The role of TE viruses in aseptic meningitis in Croatia and Slovenia
during 1961-1962:

The incidence of tickborne encephalitis (TE) virus infections in clinical patients in Croatia and Slovenia has been investigated by cooperating laboratories in the course of the last few years. TE virus infections have been known in Slovenia since 1953; sporadic cases have also been found in Croatia. Geographical distribution of serologically identified cases of these infections in the northwestern part of Yugoslavia during 1961-1962 is presented in figure 1. It is evident that there are two endemic areas: one, known since 1953, in the central part of Slovenia; and the second, recognized in 1960-1961, in the western part of Croatia. There were also sporadic cases of infections on the Adriatic coast and islands.

Special attention has been directed toward the role of the TE viruses in producing affections of the central nervous system, which were characterized in the majority of patients by aseptic meningitis. More severe forms with paretic and paralytic cases were noted. Virus isolation attempts from clinical specimens of some cases were made in primary human amnion cells, and in suckling mice. Sera were tested by complement fixation test (CF) against: 1) the three types of living, virulent poliovirus antigens prepared in FL cultures; 2) pooled adenovirus types 3, 4, and 7 antigen prepared in HeLa cultures; 3) mumps antigen produced in embryonated eggs; and 4) acetone-ether extracted suckling mouse brain antigen for the TE virus. The same type of antigen was used for the HI test. These antigens were produced by cooperating laboratories.

Specimens from 395 patients with aseptic meningitis in Slovenia were tested during 1962. There were approximately 130 examinations in Croatia for both 1961 and 1962. The number of cases reported to the epidemiology departments was much higher, i. e., 1043 for Slovenia in 1962; no data for Croatia was available. Immunologic identifications of enteroviruses isolated during 1962 demonstrated marked differences

between the two republics. In Croatia, there were 17 strains of Coxsackie B viruses belonging to type 1, 3, and 5, and 4 Coxsackie A viruses out of the 26 isolates. Slovenian results showed only one strain of Coxsackie B type 5 virus, while the remainder was immunologically related to the ECHO virus group (types 1-8, 6, 16). It is of considerable interest that no polioviruses were found.

Seasonal and age distribution of cases of the TE and enteroviruses are shown in figures 2 and 3. There were approximately 13% per cent of aseptic meningitis cases in Croatia during 1961 and 1962 caused by TE viruses; the percentage of these infections in Slovenia was as high as 32.4 per cent during 1962.

Comparative serological investigations by means of CF and HI tests were made in both laboratories. The results are presented in figures 4, 5, and 6. There is some evidence that CF antibodies against the TE virus appear later than HI antibodies in the course of the disease. It was especially noted in the Slovenian experience that fourfold or greater CF antibody rise in paired sera was obtained more frequently than in Croatia. It must be mentioned, however, that most of the sera tested in the Croatian laboratory, were obtained after the first ten days of the disease. It has also been observed that in some cases CF, like HI, antibodies may persist in some cases for long periods after primary sensitization. Before a definite conclusion might be expected, further investigations must be performed on the persistence of antibodies or the possible role of repeated contacts with the virus in endemic areas.

In countries where living, attenuated poliovirus vaccines of Sabin and Koprowski are used on a large scale, it is important to evaluate the etiologic role of TE viruses in cases of aseptic meningitis.

FIGURE 1

**GEOGRAPHICAL DISTRIBUTION OF SEROLOGICALLY IDENTIFIED CASES OF TE
IN CROATIA AND SLOVENIA**



FIGURE 2

SEASONAL DISTRIBUTION OF SEROLOGICALLY CONFIRMED CASES OF THE T. E. VIRUS INFECTIONS, AND OF PATIENTS FROM WHICH ENTEROVIRUSES WERE ISOLATED IN CROATIA (1961-1962) AND SLOVENIA (1962)

Month	CROATIA				SLOVENIA	
	T. E.		Enteroviruses		T. E.	Enteroviruses
	1961	1962	1961	1962	1962	1962
January						
February						
March						
April			1		4	
May	8	3			13	
June	6	9	1		22	
July		3	4	4	31	
August	1	3	5	9	29	5
September			1	13	16	8
October					9	8
November					4	4
December	1					1
Total	16	18	12	26	128	26

FIGURE 3

AGE DISTRIBUTION OF SEROLOGICALLY CONFIRMED CASES OF THE T. E. VIRUS INFECTIONS AND OF PATIENTS FROM WHICH ENTEROVIRUSES WERE ISOLATED IN CROATIA (1961-1962) AND SLOVENIA (1962)

Age group (years)	CROATIA				SLOVENIA	
	T. E.		Enteroviruses		T. E.	Enteroviruses
	1961	1962	1961	1962	1961	1962
0-5			5	7	2	10
6-10	2	1	5	8	11	8
11-20	1	3	2	5	22	8
21-30	4	5		4	33	
31-40	7	4		2	20	
41-50	1	3			17	
51-60	1	1			13	
61-70		1			9	
71-80					1	
Total	16	18	12	26	128	26

FIGURE 4

COMPLEMENT-FIXING AND HAEMINHIBITION ANTIBODIES AGAINST TE VIRUS IN SERA FROM SEROLOGICALLY IDENTIFIED CASES IN SLOVENIA DURING 1962

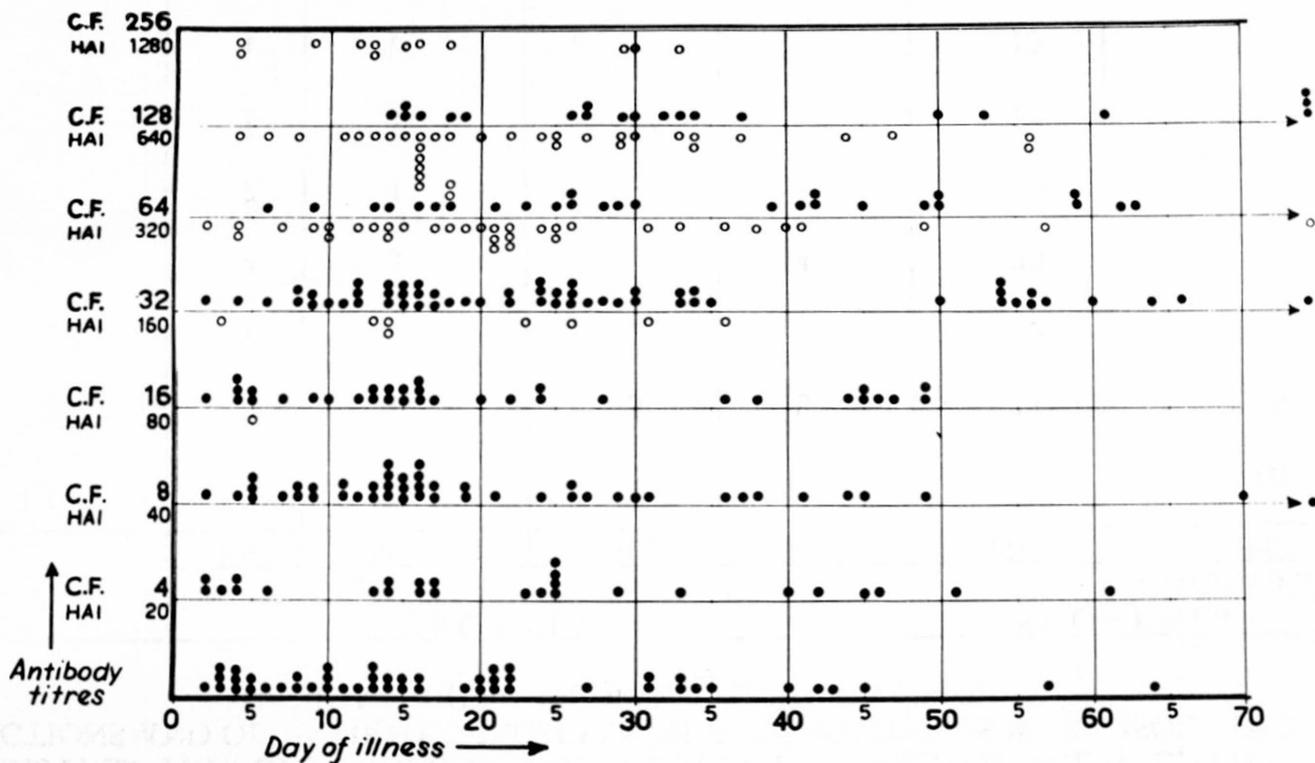


FIGURE 5

COMPLEMENT-FIXING AND HAEMINHIBITION ANTIBODIES AGAINST TE VIRUS IN SERA FROM SEROLOGICALLY IDENTIFIED CASES IN CROATIA DURING 1961 & 1962

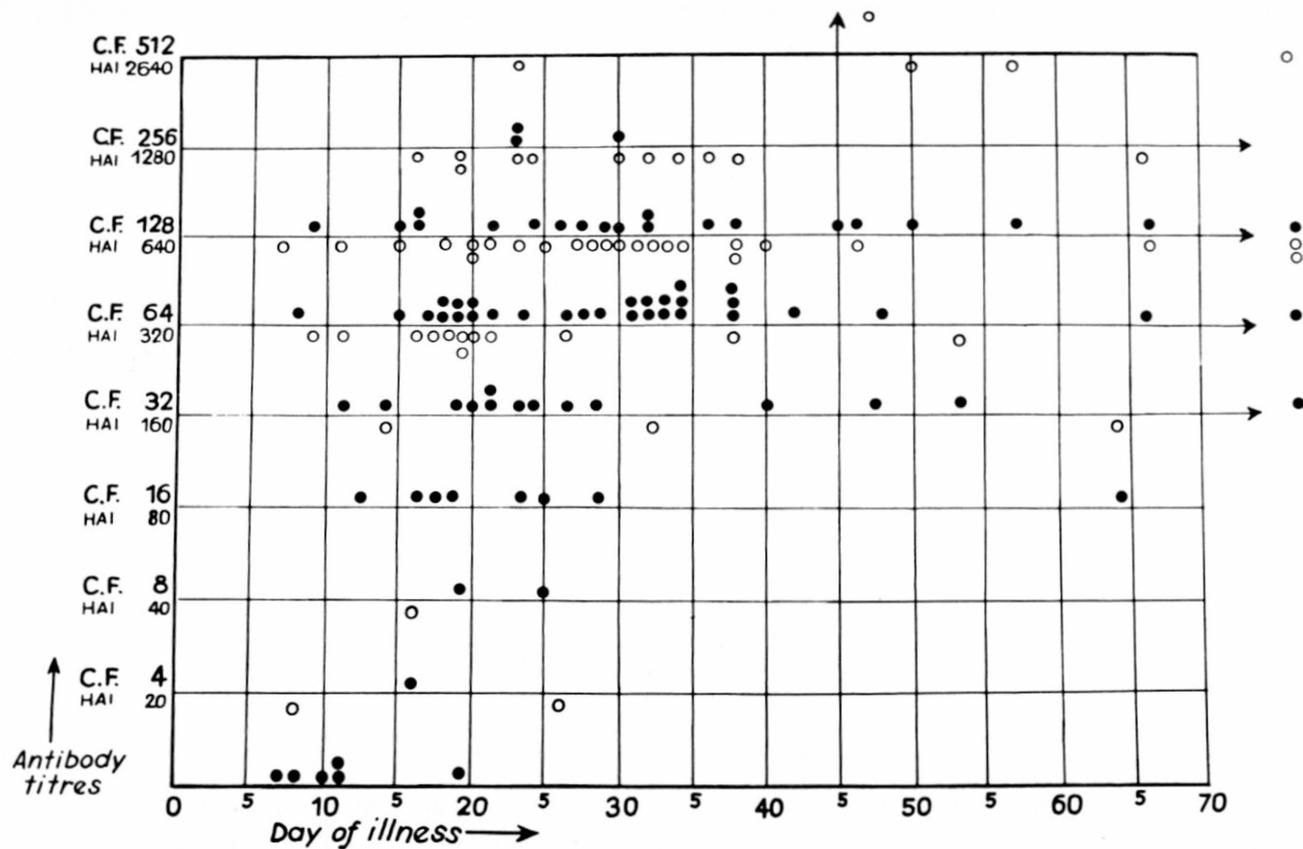


FIGURE 6

COMPLEMENT-FIXING AND HAEMINHIBITION ANTIBODY TITERS AGAINST THE
T. E. VIRUS IN SOME PATIENTS WITH SEROUS MENINGITIS

No. of Patients Examined	No. of Sera Tested	Negative*	Conversion**	No change In Titer	ANTIBODY RISE		
					2x	4x	4x
Slovenian Results: 1962							
39 CFR	106	5 12.82%	3 7.69%	2 5.1%	6 15.4%	10 25.6%	13 33.3%
39 HAI	106			12 30.7%	17 43.5%	7 17.9%	3 7.69%
Croatian Results: 1962							
18 CFR	43	2 11.1%		6 33.3%	4 22.2%	3 16.6%	5 27.9%
18 HAI	32			10 55.5%	6 33.3%	2 11.1%	
1961							
16 CFR	40			4 25.0%	6 37.5%	1 6.25%	5 31.2%
14 HAI	26			3 21.4%	5 35.7%	1 7.1%	5 35.7%

*Negative in starting dilution of serum 1:4 (CFR), 1:20 (HAI)

**Convalescent serum positive in starting dilution of serum only

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

Increasing awareness of the significance of biological and structural differences in Argas ticks, and their relationships to virus transmission, has led to comparative study of Argas parasites of birds around the world and most recently to evaluation of Argas persicus populations from domestic fowl and wild birds. It is now obvious that actually Argas persicus consists of a complex of closely related species in different areas of the world and that several of these species are associated with wild birds. Thus far, all samples that have been examined from domestic fowl appear to represent a single species, the true Argas persicus. Separate species in this group from Asia, Africa, and North America are under study. Where living material is available, biological differences between wild and domestic bird parasites of the persicus group are proving to be at least as distinctive, if not even more so, than structural differences between species. While it appears possible that wild and domestic birds may typically not share the same persicus group parasite, several recent Russian studies advance circumstantial evidence for the exchange of Argas persicus (and pathogens) between wild birds and fowl in new state farms.

In collaboration with the University of Helsinki, it was found that of 2,619 migrating birds examined on arrival in Finland during the spring of 1962, 56 were infested by 99 larvae and nymphs of the European-Asiatic tick Ixodes ricinus. Hosts were Parus major, Turdus pilaris, T. ericetorum, T. musicus, T. torquatus, T. merula, Phoenicurus phoenicurus, Luscinia svecica, Erithacus rubecula, Sylvia atricapilla, S. communis, Prunella modularis, Lanius collurio, and Phylloscopus trochilus. Two birds, Emberiza hortulana and Anthus trivialis, were infested by 11 nymphs of the European-Asiatic tick Hyalomma m. marginatum (H. p. plumbeum of Russian authors).

Ixodes ricinus carried by migrating birds may be responsible for the scattered occurrence of louping ill antibodies in northern Finland, where established populations of this tick are unknown.

In the environs of Oula City, where 1,396 breeding (non-migrating) birds were examined, ticks were found only on 9 (out of 674) Sand Martins, Riparia riparia. These ticks, Ixodes plumbeus, a reservoir of RSSE virus in Russia, have not previously been recorded from Finland.

Study of autumn migrants in Egypt during 1962 added five more species to the list of 40 bird species recently reported as tick-infested during this passage. A few specimens of previously unencountered tick species were taken during 1962; these were Ixodes redikorzevi, Haemaphysalis inermis, Hyalomma dromedarii, H. a. anatolicum, and H. anatolicum excavatum. The prevalence of tick infestation of many bird species in 1962 was considerably higher than any previous records.

During the 1962 spring migration in Egypt, a number of species not previously found to be tick infested during this passage were carrying ticks, as well as four other species that were either not previously infested or had not been examined before 1962. Spring studies were done on the Mediterranean coast near Alexandria in 1962; not in the Cairo area as in earlier years. Most ticks were Hyalomma marginatum rufipes with fewer numbers of Amblyomma variegatum and Ixodes sp. (?nov.).

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

The serological survey for arboviruses in French-speaking West Africa has now been completed in the Republic of Haute-Volta, following the completion of the study in Senegal.

To this purpose, 694 sera have been collected for examination, first by HI tests, and if necessary, by NT tests. At the beginning of this work, the results of HI tests with 120 sera already give a first impression which is interesting for the situation in that part of Africa:

Percentages of IHA positive

Age	Chik	O'N-n	Yf	Zika	Ug S	W-N	Bun	Total
1-20	78.5	85.7	59.2	42.3	31.4	33.3	07.8	56
21-50	60.3	86.7	83.0	83.0	62.2	75.0	09.4	53

As can be seen, group A immunity is very frequent and reaches approximately the same percentages before and after 20 years. This well may be the proof of a recent epidemic, as 11 sera are positive and 4 negative in the 1-4 age group. The antibody level is four times higher

with O'N-n than with Chick antigen in 41 sera. The reverse occurs only in 5 cases, so O'N-n would seem to be the causative agent.

In group B, antibodies are also frequent, but more in the second age group than in the first. This would mean repeated infections as people grow up. The situation for Yf is a special case, as a program of mass vaccination of every four years has been followed in this country since 1939.

Group Bun is at a very low endemicity without age prevalence, but the only Bun strain used may not be representative enough for the group. This serological pattern is not so different from that of Senegal; nevertheless, immunity against Group A is more important and more recent.

Since the isolation of the first strains of arboviruses in the fall of 1962 by this laboratory, we have gone to the East Africa Research Institute (Dr. Haddow) at Entebbe, Uganda, to check our results.

A strain obtained from a sentinel suckling mouse, which had been exposed in a forest, which showed a close relationship to O'N-n, has been confirmed. Another strain isolated from insectivorous bat's salivary glands (P. Bres et L. Chambon, 1963, Ann. Inst. Pasteur, 104, pp. 705-711) is perhaps a new group B virus related to H 336 but not to Entebbe Bat II 30. Two more strains isolated from bat's salivary glands' pools behaved identically to each other and were identified as strains of Chick but with no HI cross with O'N-n, a fact which is unusual.

This emphasizes the role played in West Africa by insectivorous bats, which are very numerous, and indicates a need for a more extended study for investigating the vectors which are in relation to them. This points out also the frequency of strains of group A.

REPORT FROM DR. J. DEMARCHI
PASTEUR INSTITUTE OF BRAZZAVILLE,
REPUBLIC OF THE CONGO

Two Pasteur Institutes are located in Equatorial Africa, the Pasteur Institute of Brazzaville (Republic of the Congo) and the Pasteur Institute of Bangui (Central African Republic).

The activity of these two institutes is coordinated under the authority of an agent of the Pasteur Institute in Equatorial Africa who assumes likewise the functions of the director of the Pasteur Institute in Brazzaville.

I. The Pasteur Institute of Bangui, completed in 1961, was conceived and set up with a view to the study of the epidemiology of the arboviruses in the region of Central Africa. The program now being developed includes the following points.

A. Delineation and study of the natural ecological zones making up the region. This study includes in particular the naming, identification, the estimation of the relative importance, on one hand of the potential vectors, and on the other hand, the mammals and birds as potential reservoirs of arboviruses or in its natural cycle.

B. In the interior of the defined zones, serological surveys involving the human population and eventually the animal population will be carried out by means of the classic HI test. The antigenic "keyboard" used has been determined provisionally as a function of the data already established by the E.A.V.R.I. of Entebbe (Chikungunya, Sindbis, West Nile, Bunyamwera, and yellow fever, to which we will add this year ONN, Zika, and Uganda S).

Currently, particularly interesting points are being furnished by serological study of domesticated bovines which move from the hills across the western zone of the region.

C. When these first investigations have been accomplished, trials for isolation of arboviruses from animals and vectors will be undertaken.

II. The activity of the Pasteur Institute of Brazzaville has been directed toward studies of nutritional biochemistry and on host-parasite relationships involving trypanosomiases. It was only at the beginning of 1963 that an arbovirus department was initiated. This laboratory must on the one hand serve for the standardization of tests used at the Pasteur Institute of Bangui, and on the other hand, apply to arboviruses the technics used in other fields (adsorption chromatography, double-diffusion, immunofluorescence).

REPORT FROM ARBOVIRUS RESEARCH UNIT
SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH
JOHANNESBURG, REPUBLIC OF SOUTH AFRICA

Sindbis virus infections in man:

Last summer, several cases of illness, suggestive of arbovirus infection in persons resident in the South African highveld, were encountered. As reported in the previous Infoexchange, Dr. Malherbe of the South African Institute for Medical Research isolated Sindbis virus from one such case. Although virus was not isolated from further cases, paired sera were available and at least 12 of these cases were diagnosed on serological evidence as being due to Sindbis virus. The table shows the antibody results on paired sera from 5 of these cases. A diagnosis of Sindbis appears justified on account of the appearance or rise in Sindbis antibody during convalescence, while antibody against the other three Group A viruses known to occur in South Africa was absent.

The illness was of 4-8 days duration and the following symptoms occurred: lassitude, slight headache, slight fever, sore throat, enlarged glands (rare), muscle and tendon pain, joint pain which sometimes persisted for several weeks, maculo-papular rash, which sometimes itched, on trunk and limbs. In some cases, vesicles developed between the fingers and toes. One case showed symptoms of myocardial involvement. In support of the diagnosis of Sindbis, it should be mentioned that this virus was isolated 8 times from mosquitoes and once from a wild bird collected at 2 localities on the highveld last summer.

Chikungunya:

During March 1963, the area in Southern Rhodesia involved in the outbreak of chikungunya in 1962 was revisited. It was hoped that mosquito collections at the height of the mosquito season would indicate the likely vector during the epidemic, as our visit in 1962 was too late in the season for worthwhile observations in this respect. One thousand, three hundred and seventy-six of the tree-hole breeding Aedes (Diceromyia) furcifer and/or A. taylori were collected. The females of these species are indistinguishable, but examination of terminalia from wild-caught males showed that both species were present although furcifer appeared to be the more prevalent species. These mosquitoes fed readily on human

bait for about an hour soon after darkness. At this time, they were collected in a tree 20 feet from the ground, on top of a granite koppie, where a troop of baboons habitually spent the night, and on an anaesthetized monkey. A number of female A. furcifer/taylori were brought back to the laboratory for transmission experiments. These showed a high vector capability with chikungunya virus. The mosquitoes were infected by feeding on viremic monkeys (6.2 and 7.2 logs) and transmissions were successful to mice on the 7th and 13th days and to a monkey on the 19th day. While feeding the mosquitoes on this monkey in a darkened room, two persons were apparently accidentally bitten by infected mosquitoes. Both contracted chikungunya. Between 30 and 36 days after their infective feed, 14 surviving mosquitoes were tested individually for virus. Thirteen were positive. Eggs were obtained from some of the mosquitoes and of the 7 adult males obtained from these eggs, all were furcifer. While furcifer would appear to have been definitely involved in the successful transmissions, a certain number of taylori may also have been present.

As mentioned in a previous Infoexchange, we failed to transmit chikungunya virus with Anopheles gambiae. However, in view of the prevalence of Anopheles in our collections in Rhodesia this year, another attempt was made to transmit chikungunya with Anopheles gambiae. Thirty An. gambiae engorged on a viremic monkey (7.2 logs). Transmission to a monkey 19 days later failed. Of 11 mosquitoes tested for virus between the 2nd to 36th days, only one was positive.

Blood specimens were collected from 9 vervet monkeys and 4 baboons trapped at Chipinda Pools and Lone Star Ranch where human cases occurred in 1962. HI tests on these sera showed all these animals to be positive for chikungunya virus antibody.

On grounds of its habits, vector capability and prevalence, it appears very likely that A. furcifer/taylori was deeply implicated as a vector in the 1962 outbreak. Although not a true domestic mosquito, the siting of African huts in amongst the bush and the habit of the mosquito to feed in the early evening before the occupants retire, would provide ample opportunity for exposure of man. It also appears likely that this mosquito is an important feral vector, involving wild primates, and would also act as a link between feral cycles and man.

Antibody Tests on Sera from Persons Suspected of Being Infected With Sindbis Virus

Case	Serum	Days after onset	Antibody Results With					
			Sindbis			Chik.	Midd.	Ndumu
			HI	NT	CF	HI	HI	NT
1	1	5	80	N	N	N	N	-
	2	14	1280	P	N	N	N	N
2	1	6	160	N	N	N	N	-
	2	15	1280	P	16	-	N	N
3	1	3	20	N	N	N	N	-
	2	26	640	P	4	N	N	-
4	1	5	N	IC	-	N	N	-
	2	38	640	P	4	N	N	N
5	1	4	80	N	N	N	N	-
	2	30	640	P	32	N	N	N

P = positive; N = negative; - = not tested; IC - inconclusive

REPORT FROM DR. C. A. MIMS,
AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA,
AND DR. M. F. DAY, C. S. I. R. O., CANBERRA, AUSTRALIA

Cytopathic effect of semliki forest virus on its vector:

A marked cytopathic effect on the salivary glands of Aedes aegypti was observed 14, 21, but not 7, days after infection with Semliki forest virus. The CPE was observed when the source of virus was suckling mice with a viremia of 10^9 pfu per ml, but not when the source was adult mice with a viremia of 10^5 pfu per ml.

Cytopathic changes were most pronounced in acinar cells of lateral and central lobes of the glands. No organs other than salivary glands were affected. Salivary glands of Aedes scapularis malayensis were not affected.

Aedes aegypti transmission of SFV is unusual in that the vectors lose the ability to transmit about 3 weeks after infection. It is suggested that the CPE and loss of infectivity may be related. This is the first report of a CPE on the tissues of a vector of an arbovirus.

REPORT FROM DR. IAN D. MARSHALL, MR. ROYLE A. HAWKES,
AND MR. KEN LAM, DEPARTMENT OF MICROBIOLOGY,
AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA

A linear study of a group of native children under the age of six years has been undertaken to obtain information on arbovirus incidence and activity in the Sepik District of New Guinea. The base-line sampling was carried out in April 1963, the villages being selected from the various ecological situations described in the March 1963 Infoexchange. In general, the incidence of HI antibodies (Table 1) is a reflection of observed mosquito activity in the various areas (March 1963 Infoexchange), there being a pronounced increase of presumed arbovirus circulation over the thirty miles between the foothill villages and those on or near the Sepik River. A second sampling of the same children has just been completed and early results from the river villages indicate that there have been a number of conversions and boosted titers during the four months interval. Mosquitoes collected during this interval have yet to be tested.

Although the relatively high incidence of antibodies in the under one-year-olds has been attributed to maternal origin, it is not clear why this incidence is higher in the foothill group than the river group. In addition to tracing out the fate of these antibodies in subsequent samples, a number of mothers have been bled to find whether their antibodies match those of their babies.

To date, the sera have been tested against Semliki Forest virus, Sindbis, N544, (Group A) Murray Valley encephalitis (Group B), and Wongal (Koongol group). The latter antigen reacted with only one serum, and that from a three-month-old baby. Of the group A antigens, SFV produced most reactions in sera from river villages, while N544 (Getah virus) was more common away from the river.

Although the incidence of antibodies in children could be predicted from the varying mosquito density and attack rates in the different areas, preliminary surveys indicate that antibodies in wildlife appear to be more evenly distributed between the foothill and river regions. The reasons for this discrepancy are not at present apparent.

TABLE 1
 HI ANTIBODY - SEPIK DISTRICT, NEW GUINEA - APRIL, 1963

Age Group	Foothills				Peripheral Plains				Plains				River				Totals								
	No.	Group A		Group B		No.	Group A		Group B		No.	Group A		Group B		No.	Group A		Group B						
		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%	No.	%			
3 months to 1 yr. (Maternal ab)	56	1	1.8	10	17.8	14	0	0	1	7.1	19	1	5.2	1	5.2	16	1	6.2	1	6.2	105	3	2.9	13	12.1
1 - 2 yrs.	75	1	1.3	0	0	43	2	4.6	2	4.6	23	3	13	1	4.3	12	0	0	1	8.3	153	6	3.9	4	2.6
2 - 4 yrs.	170	2	1.2	1	0.6	88	4	4.5	2	2.3	45	4	8.9	1	2.2	23	5	21.8	4	17.4	326	15	4.6	8	2.4
4 - 6 yrs.	73	5	6.8	1	1.3	60	6	10	4	6.6	25	5	20	4	16.0	38	11	29.0	14	36.8	196	27	13.8	23	11.7
Totals	374	9	2.4	12	3.2	205	12	5.9	9	4.4	112	13	11.6	7	6.2	89	17	19.1	20	22.4	780	51	6.5	48	6.2

REPORT FROM THE ARTHROPOD-BORNE VIRUS RESEARCH UNIT,
LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

Encephalitis in Sarawak:

Dr. C. E. Gordon Smith, with Dr. H. E. Webb of St. Thomas's Hospital and Mr. W. W. McDonald of the Liverpool School of Tropical Medicine, spent December 1962 and January 1963 in Sarawak. The records of the General Hospital, Kuching, for 1959, 1960, and 1961 revealed 65 cases of encephalitis mainly in children. The cases seen clinically resembled those elsewhere caused by arthropod-borne and enteroviruses. A temporary laboratory was established near Kuching: mice were air-freighted from Kuala Lumpur and dry ice from Singapore. Much of the initial mouse inoculation for virus isolation was done in Sarawak. Specimens and sera were later flown to London for further study. Satisfactory pairs of acute and convalescent phase sera were obtained from 14 encephalitis patients. Seven of these showed diagnostic increases in haemagglutinin-inhibiting (HI) and complement-fixing (CF) antibody to a group B arbovirus related to Japanese encephalitis and Tembusu (MM 1775) viruses. Another 4 were possibly also group B infections. Virus isolations were attempted from 36 acute-phase blood samples from febrile patients: a virus, probably dengue, was isolated from 1. Satisfactory paired sera were collected from 28 patients with febrile illnesses: in addition to the probable dengue patient, 2 others showed antibody responses typical of group B infections. Three were found by Dr. L. H. Turner to be cases of leptospirosis.

A total of 862 human sera from a variety of places, mainly from younger age groups, and 50 pig sera, were collected for serological surveys. Further study of the Sarawak sera collected in 1961 has shown that group A arbovirus infections are much less common than group B infections. The high prevalence of HI antibody to certain group A viruses previously reported was apparently due to the use of redistilled acetone for serum extraction.

A total of 20,574 mosquitoes of about 90 species were hand-caught in tubes coming to bite man at 4 main sites and in a number of minor collections. A total of 2,789 were taken in a pig-baited Magoon trap at a piggery. In all, 18,911 mosquitoes were ground up in 170 pools of about 100 and inoculated into suckling mice. So far, there are 3 definite isolations (and reisolations) of virus: MS50 from Aedes

(Cancraedes) curtipes; MS117 and MS128 from Culex tritaeniorhynchus. All were from mosquitoes taken at Kampong Pangkalan Kuap. A. curtipes was the commonest day-biting mosquito in the village itself. The C. tritaeniorhynchus were taken at the ricefields about a mile away through secondary forest.

Some mosquito species were very common in one area but absent or rare in the others, for example, A. curtipes in Kg. Pangkalan Kuap and Aedes (Skusea) amesii/fumidus in a nipah palm area. Some, on the other hand, were present to a greater or lesser extent almost everywhere; foremost among these were the day-biting Aedes (Stegomyia) albopictus and the night-biting C. tritaeniorhynchus and Mansonia annulifera.

MS117 and MS128 are apparently identical and are probably strains of Tembusu virus. Further studies are in progress to identify the 4 viruses isolated and to determine whether the encephalitis cases were due to MS117/128.

Louping ill: Studies of sheep have continued in collaboration with Mr. A. L. Wilson of the Veterinary Investigation Department for the West of Scotland and Mr. K. J. O'Reilly of the Wellcome Research Laboratories. The prevalence of louping ill in sheep was low in 1962. The studies of ticks and small vertebrates in the same farms with Dr. M. G. R. Varma and financial assistance from the Agricultural Research Council have also continued. A total of 130 rodents and insectivores were trapped in March/April, 1963, and are being studied for evidence of infection. Tick collections are being made on drags and on sheep throughout the year so as to relate tick population and activity to vegetation patterns and infection rates.

As 1959 was a vole-year and 1960 an epizootic year in lambs and because the disease occurs in such epizootics at intervals of years, it is suggested that years of high prevalence are related to the population of small vertebrates in the preceding year. It is hoped to continue these studies at least until the next epizootic year so that a full picture of the epidemiology can be built up.

Mr. Chan Yow Cheong, Wellcome Fellow in Tropical Virology, has been carrying out a detailed study of the antigens of louping ill virus by calcium phosphate chromatography and density gradient centrifugation. Hemagglutinin, complement-fixing, and precipitating antigens are being

measured and compared. Different strains of louping ill virus give different and characteristic fractionation patterns by these methods.

Miss Fatima Begum has been studying the growth, heat stability, antigens, and chromatography of plaque-purified lines of louping ill and Langat strains with different passage histories.

Langat virus:

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

REPORT FROM DR. Y. KANDA INOUE, INSTITUTE FOR VIRUS RESEARCH, KYOTO UNIVERSITY, KYOTO, JAPAN

Selection of a Clonal Line of Porcine Kidney Stable (PS) Cells for Cultivation of Japanese Encephalitis Virus (with co-investigation of Dr. Masa-atsu Yamada):

The alteration of susceptibility of PS cells serially cultivated in different media have been experienced. Propagation of PS cell populations from single clones isolated by the Puck's technic provides cells which are more stable in growth potential and virus sensitivity than parental populations from which they were selected. Populations from 8 clones have been studied and it was observed that a clone (Y-15) was the most sensitive to JE virus. The growth medium for PS cells consisted of 10% calf serum and 0.5% lactalbumin hydrolysate in Earle's balanced salt solution, and the trypsinization has been done with the mixture solution of trypsin and Versine. It will be a pleasure to send PS cells (Y-15) to my friends in other arbovirus laboratories.

References: Virology (1962), 16:205, and 18:500.

An Attenuated Mutant of Japanese Encephalitis Virus:

An attenuated mutant (m mutant) was isolated after serial passages of a parental virus, Mukai strain, in embryonic mouse skin cultures. The low virulence of the m mutant was stable in tissue cultures but reversion to virulence was observed after multiplication of the virus in mouse brain. The m mutant induced high immunity in mice after single intraperitoneal vaccination. Furthermore, a high virus yield was obtained from the m mutant in tissue culture with titers of about $10^{9.5}$ TCD₅₀ per ml. It is suggested that the m mutant will be suitable for the preparation of inactivated tissue culture vaccine. (Details of this investigation will be published in Bull. Wld. Hlth. Org., 1963).

A Thermo-efficient Mutant of Japanese Encephalitis Virus (with co-investigation of Dr. H. Kato):

A thermo-efficient mutant (rct/42⁺ mutant) characterized by its reproductive capacity at 42°C was isolated from the wild type (rct/42⁻) JE virus. The rate of thermal inactivation of free virus particles of the rct/42⁺ mutant was the same as that of rct/42⁻ virus. Plaques produced by rct/42⁺ virus at 36°C were minute and usually uncountable, whereas rct/42⁻ virus produced plaques of 4-6 mm in diameter. The intracerebral titer of the rct/42⁺ mutant for young adult mice was nearly the same as the tissue culture titer obtained at 41°C. The virulence of the rct/42⁺ mutant for young adult mice following intranasal inoculation was greater than that rct/42⁻ virus. (Details of this investigation will be published in Virology, 1963).

REPORT FROM DR. S. HOTTA, DEPARTMENT OF MICROBIOLOGY
KOBE MEDICAL COLLEGE, KOBE, JAPAN

In vitro cultivation of culicine tissues (S. Kitamura):

1) Sterile cultivation of culicine mosquitoes:

Two or three rafts, collected in a test tube, were washed by pipetting, three times with 0.25% HgCl₂ solution in 80% alcohol, then three times with sterile distilled water. Culture medium consisted of 4% dried yeast powder (autoclaved), 0.05% dried yeast powder (filter-sterilized) and 0.1% lactalbumin hydrolysate (filter-sterilized). About 30 sterilized eggs were inoculated into a 250 ml glass bottle containing

50 ml culture medium, which was then incubated at 22-24°C. When the first instar larvae appeared, each bottle was adjusted to contain 15 individuals, and was re-incubated. Usually it took about 2 weeks for complete development of adults.

2) In vitro cultivation of midgut tissues of mosquitoes, Culex pipiens var. pallens:

Constituents of basal medium (g per liter) were: NaCl (8.5), KCl (0.3), MgCl₂·6H₂O (0.1), CaCl₂ (0.02), NaHCO₃ (0.1), KH₂PO₄ (0.2) and Glucose (0.8). Culture medium consisted of 70 ml basal medium, 10 ml alcoholic larvae extract, 10 ml yeast extract 1% solution, 10 ml lactalbumin hydrolysate 1% solution, and 100 mg glutamate.

Midgut tissues, removed aseptically from the sterile-raised 4th instar larvae, were cultivated at 22°C by the hanging drop method using the culture medium above described. Giemsa staining and phase contrast microscopy were performed for morphological observations. Several types of cells were shown to migrate from the original tissue pieces. Some of them were: large circular cells containing a fine nucleus and a number of granules; small spindly cells; cells with fine projections; cells of epithelial nature, etc. The majority of the cells were maintained well for about 60 hours without changing the culture medium, and for 2 to 3 weeks by changing the culture medium.

Improvements of the culture techniques are being tried.

REPORT FROM MAJOR SCOTT B. HALSTEAD,
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SCHOOL OF PUBLIC HEALTH, BANGKOK, THAILAND

Thai hemorrhagic fever (THF):

The epidemic of 1962 has been the biggest in the history of Thailand. Hospital admissions alone totaled 3,774 among residents of Bangkok and Thonburi and 2,312 among residents of major towns in the Central Plain and the Southeast and Southwest coastal regions along the Gulf of Siam. Attack rates in Bangkok were 18 per 10,000 while in some of the up-country

outbreaks, rates as high as 193 per 10,000 were observed. Age specific attack rates and death rates in 1962 in Bangkok and in communities which never reported the disease before were similar to rates of previous outbreaks in Bangkok, i. e., cases and deaths were confined almost entirely to children below the age of 13; the case fatality rate was 5.6%. Bangkok is unquestionably an endemic focus for dengue viruses since dengue virus confirmed hemorrhagic fever admissions now have been seen in every month of the year. For reasons that are obscure, the disease did not spread into the northeastern and northern sections of the country although towns in these areas are serviced by good rail and road connections with Bangkok and have high Aedes aegypti populations. In other respects, however, the epidemic followed the known distribution of Aedes aegypti virus. That is, cases came from towns and cities and not from isolated villages or farm houses.

As a result of an intensive area survey at Bangkok during the epidemic, it has been estimated that there were 3,790 moderately severe illnesses identified as THF by physicians and treated in private hospitals or at home. In addition, approximately 151,000 children in Bangkok and Thonburi had milder illnesses caused by dengue or chikungunya viruses which were treated by physicians. These illnesses were largely manifested by URI symptoms.

Nearly 50 Caucasians had laboratory confirmed illnesses with dengue and chikungunya viruses in Bangkok in 1962. Most of these were in adults; children's illnesses were mild and no patient of any age was seen with shock or hepatomegaly, the cardinal signs of THF. It seems very likely that racial-genetic factors control the pathogenetic differences between infection in Asians and Caucasians since economic and nutritional status among Thai or Chinese had no influence on the severity of disease.

Serologically, 13% of THF hospitalizations were due to chikungunya and 87% to dengue virus infection. To date, 22 dengue viruses and 10 chikungunya viruses have been isolated from THF patients. Many more viruses have been isolated from milder and atypical illnesses.

Characterization of the 1962 agents is still in progress; 33% of human and arthropod dengues appear to be type 2 (5?), and types 3, 4 and 6 have also been identified. An attempt will be made to relate dengue types to disease severity.

Aedes aegypti stands incriminated as the principal urban vector of THF (see figure 1). Thus far, of 150 pools of Aedes aegypti tested (8, 631 mosquitoes) 20 dengue viruses and 7 chikungunya viruses have been isolated for an 18% pool recovery rate. Of 753 pools of Culex pipiens quinquefasciatus (79, 453 mosquitoes) tested, only 1 chikungunya virus was recovered. Four unidentified agents have been recovered from Culex quinquefasciatus. In view of our own and McIntosh's (South Africa) inability to demonstrate transmission of chikungunya virus with the latter species, its status as a vector of this virus must stand in abeyance.

Every attempt was made during these studies to avoid laboratory contamination especially since blind passage was routine and studies were conducted on a fairly large scale. Mosquito and mouse brain diluent were dispensed into small bottles and used for only one specimen. Every 5th arthropod specimen inoculated daily was a bovine-albumin control which was blind passed to the 3rd mouse passage. In addition, every 5th clinical specimen came from a surgical patient. Both of these control measures failed to result in factitious virus isolation.

Japanese Encephalitis in Thailand:

From known neutralizing antibody conversions in domestic animals and humans over the past 2 years, it is clear that Japanese encephalitis virus or closely related agents are widely distributed in Thailand. At this point, it is of interest to point out that a proven case of Japanese encephalitis has not yet been recognized and that seasonal peak dissemination of JEV varies by as much as 5 months between 2 points in central Thailand which are only 60 kilometers separated. As many as 80% of 3 resident avian species, Blanford's bulbul, magpie robin and tree sparrow, developed HI antibody for JE virus between the months of March to June 1962 in the Bangkok area. Peak Culex tritaeniorhynchus and Culex gelidus populations in the Bangkok area occurred in July, 1962.

At the Bangphra horse farm study site located on the fringe of the Thailand Central Plain in hilly, partially forested land, peak populations of these two mosquito species occurred in November, 1962, and 5 virus isolations have been made. Three strains of virus identical with JEV have been recovered from 2 pools of Culex gelidus and 1 pool of Culex tritaeniorhynchus, 1 strain of virus closely related to JEV has been recovered from Culex gelidus and 1 chikungunya virus recovered from Culex tritaeniorhynchus. All these mosquitoes were captured in October and November 1962. (Mosquitoes were captured and identified

and viruses isolated by Dr. Sakol Rohitayadhin of the Bangphra Pasteur Institute Serum Laboratory.)

Development of a Tissue Culture System for Primary Recovery of Dengue Viruses:

A great deal of experience has been accumulated in growing dengue viruses in various primary and stable cell lines over the past 2 years. We have finally discovered a cell line which, although expensive to maintain, is as sensitive as the suckling mouse for high and low mouse passage dengue strains (types 1-6) and equally or more sensitive than the suckling mouse for primary isolation from human and arthropod material. The cell line is the stable grivet monkey kidney cell line to be described by Hopps, Berheim, Nisalak and Smadel in the Journal of Immunology later this year. All types of dengue viruses grow in these cells to high titers. The presence of virus is determined by primary CPE (type 2) or by challenge-resistance, i. e., resistance of dengue infected cells to challenge with a CPE producing virus (100 TCD 50 of polio I). HKC, stable porcine kidney (Inoue), primary monkey kidney and MK-2 (Hull) cells have not been found to be susceptible to all types of naturally occurring dengue viruses in low mouse passage and isolation attempts from human serum using HKC have failed. Particulars will be published shortly.

Miscellaneous Laboratory Studies:

Development of autointerference in suckling mouse brain infected with o'nyong nyong virus. Difficulty in adapting strains of o'nyong nyong virus to suckling mice have been reported by M. C. Williams and others of the E. A. Virus Research Unit. This difficulty appears to be caused by the high titers of autointerference factor(s) developed in mouse brain found at the usual time of harvest, i. e., when the mouse is sick at 6-8 days after inoculation. High titers of mouse lethal virus can be recovered free of autointerfering factor if mice are harvested 36-48 hours after inoculation or if high dilutions are used for passage work (10^{-3} to 10^{-5}). The time of development of autointerference in 4th mouse passage of strain SE 2559 (kindly supplied as original human virus by M. C. Williams) is demonstrated in Table 1. A considerable variation in susceptibility to o'nyong nyong also occurs from litter to litter suggesting that mouse colonies are not homogeneous with respect to sensitivity to o'nyong nyong.

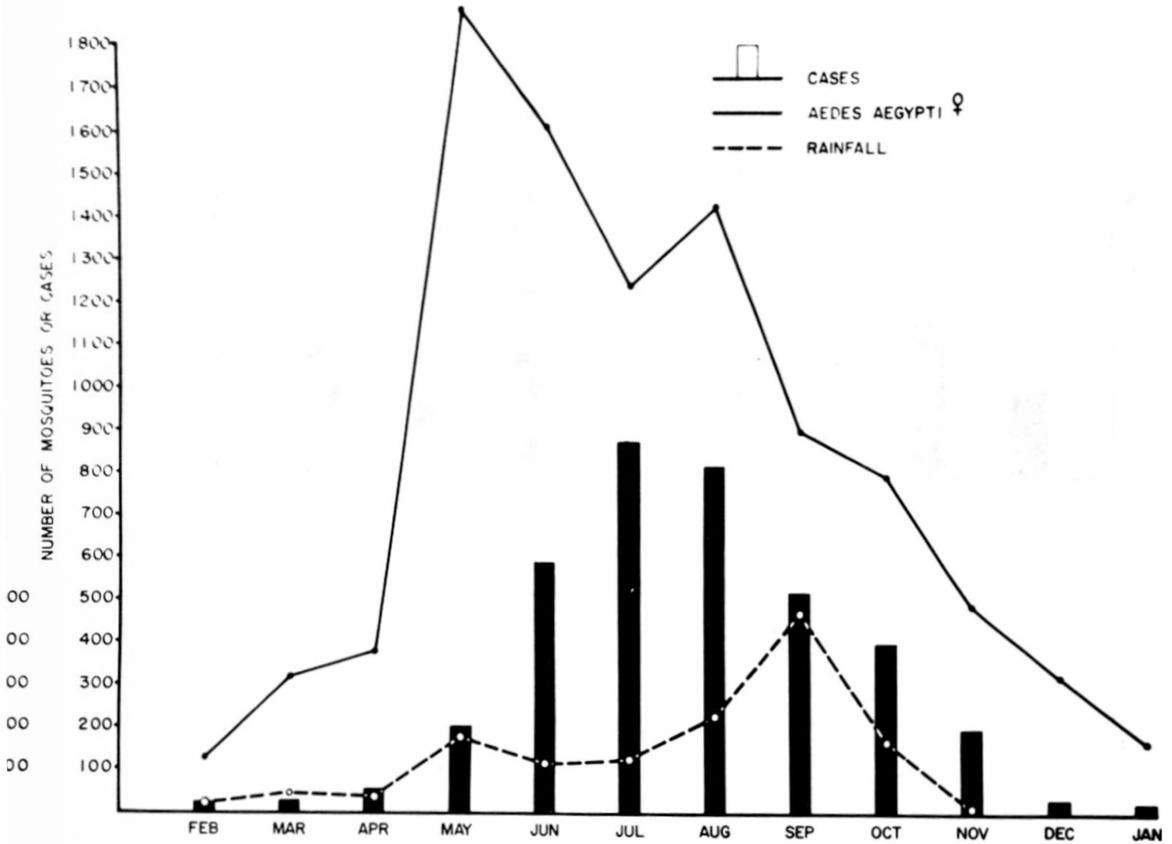
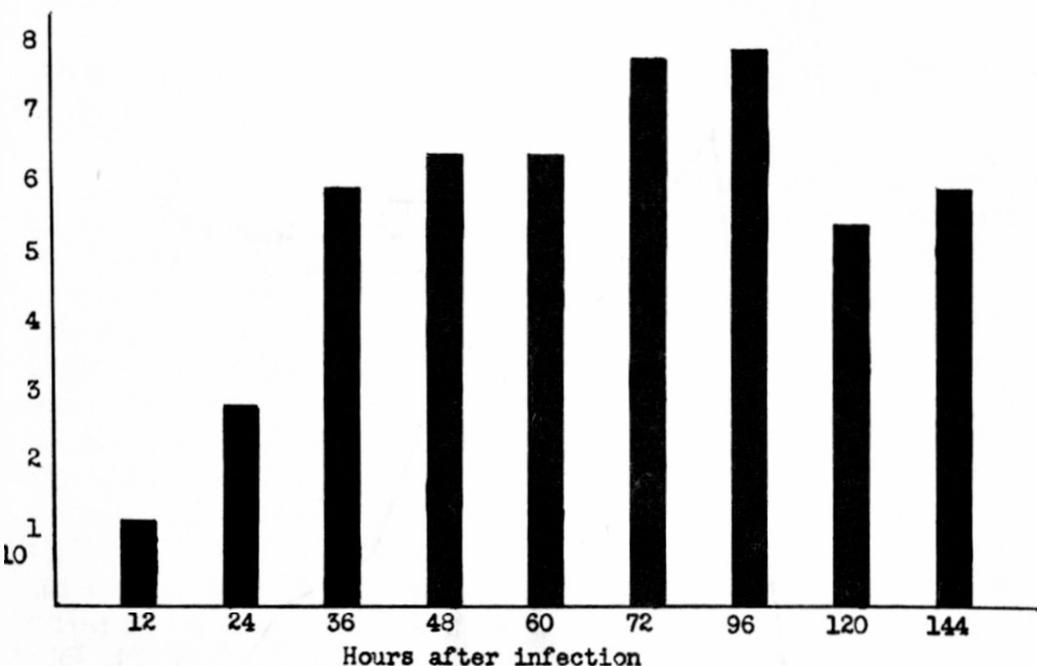


FIGURE 1 : Monthly hemorrhagic fever hospitalizations Aedes aegypti collections and rainfall, Bangkok-Thonburi, Thailand, February 1962 - January 1963.

O'NYONG NYONG VIRUS (SE 2559 SM3)

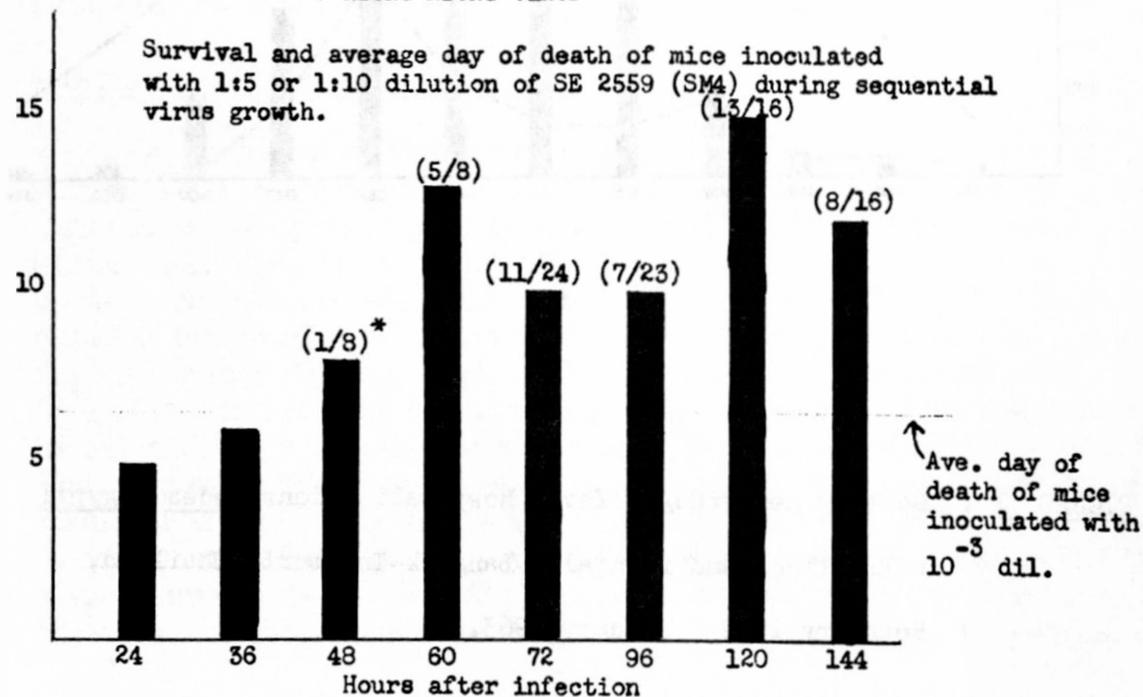
Figure 2

GROWTH CURVE IN SUCKLING MOUSE BRAIN



APPEARANCE OF AUTOINTERFERENCE IN
O'NYONG NYONG VIRUS

Survival and average day of death of mice inoculated with 1:5 or 1:10 dilution of SE 2559 (SM4) during sequential virus growth.



* Survivors shown in parentheses. For purpose of calculating ave. day of death, survivors were counted as dying on day 15.

REPORT FROM DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF OTAGO, NEW ZEALAND

New Zealand Investigations:

Further field investigations have been carried out in the area of the West Coast of the South Island where strains of a group A agent were isolated in 1962. Further strains of this virus were not isolated, but serological investigations on wild birds gave quite strong evidence of recent virus activity.

Blood plasma was obtained from 365 wild birds; of these, 102 neutralized 30 or more pfu's of the strain M 78. These sera were obtained from all over an area of 10 x 30 miles which consisted of a forested coastal plain including a large 60-year-old clearing at the foot of a big mountain chain. Immunes came from all over the area and from 13 of the 19 species of wild birds which had been sampled.

Immunity was most common amongst the song thrushes and amongst these most of the immunes (17/22) were taken in late February and March. Thirty-three of the positives were titrated for antibody content and the high titers came from orchards in the cleared area and the edge of a forested lake.

Wild birds were captured and inoculated with the virus isolated and viremia was detected in sparrows, silvereyes, thrushes and blackbirds. Titres between 10^4 and 10^6 were obtained on days 1-3 inclusive and high titer neutralizing antibody appeared by day 11 post inoculation, when a dilution of 10,240 neutralized 30 pfu's. Studies in the transmission of this virus showed that when injected into Aedes australis it multiplied rapidly and the mosquitoes were able to infect by bite. The virus survived for considerable periods in simuliids, but there was little or no multiplication.

Evidence of Human infection by a group B virus:

In the same area, and for 100 miles to the north, serological evidence was obtained of a pyrexial illness due to a group B virus occurring in the human population. Several cases had a significant rising titer of H. I. antibodies to MVE virus. The convalescent specimens did not neutralize this virus. No strains of group B virus have yet been isolated.

Sero-positive cases varied between those with influenza-like syndromes to one with a typical aseptic meningitis syndrome. The cases in which antibody was found all occurred in January or early February.

Fiji Investigations:

In 1960, a serological survey in the Fiji group of islands showed that between fifty and sixty per cent of the population aged between 20 and 40 years had neutralizing antibodies against dengue virus and that above that age the incidence rose to nearly seventy per cent. Below the age of 20 years, the incidence of neutralizing antibodies was lower, being eight per cent in the 10 to 14 years age group. These figures indicate that at least in the era preceding 1946-1950 dengue had been present in Fiji, either in an endemic state or with fairly frequent epidemics.

In January and February, 1963, further studies were undertaken to obtain evidence on the present state of endemicity of arboviruses in Fiji.

Sera were obtained from 60 patients attending a clinic in Suva. The majority of these patients complained of headache and fever. Some had enlarged cervical glands, but no exanthems were seen. The sera were injected into newborn albino mice, usually within one or two hours of collection. The remaining portion of each sample was preserved in a refrigerator at -70°C . Four strains of filterable agents have been isolated by this technique. The isolations have been confirmed by re-isolations from the original samples. These strains have a similar incubation period of three days in infant mice in which muscle necrosis is produced. They are not appreciably inactivated by sodium desoxycholate.

Nearly 2,400 mosquitoes, mostly of the species Culex annulirostris, were collected resting within houses in and around Suva. After identification, these mosquitoes were pooled in batches of 100 and subsequently ground up and inoculated into day old mice. No viruses were isolated.

To the southeast of Suva are extensive swamps associated with the delta of the Rewa river. In the mangrove forest and swamp area at the southern tip of the delta, 39,941 mosquitoes, most Aedes (Stegomyia) polynesiensis were caught on human bait. These likewise were inoculated

into day old mice. Three pools of A. polynesiensis all caught on the same day have so far yielded filterable agents. The incubation of these agents in newborn mice is three to four days.

A further 4,534 mosquitoes were caught on the islands of Vanua Levu and Taveuni to the north of the main island of Viti Levu.

The mosquito pools are still being processed for virus isolation.

All the viruses so far isolated killed mice on first passage. Further blind passages of material negative on the first passage have not yet been made.

Sera from 200 children between the ages of one and 15 years were collected for survey purposes and tested for hemagglutination inhibition antibodies against Sindbis, WEE, Murray Valley encephalitis, dengue I and II, and Japanese encephalitis virus. No inhibition was given against the viruses except against Murray Valley encephalitis virus. Against this virus in all age groups including those under three years of age about seven per cent of samples gave inhibition at a titer of 1:10 or more. The youngest individual giving a positive serum was born in 1960 and came from the vicinity of Suva. Neutralization tests are in progress.

No virus was isolated from the spleens of 70 cave bats, and the sera of two flying foxes were negative on serological examination to the viruses named above.

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TABLE I

Species	Totals	Positives	Inconclusive	Negative	% Immune
Blackbirds (<u>Turdus merula</u>)	148	40	3	105	27%
Song thrushes (<u>Turdus ericetorum</u>)	43	22	1	20	48%
House sparrows (<u>Passer domesticus</u>)	51	15	1	35	29%
Silvereyes (<u>Zosterops lateralis</u>)	74	10	1	63	14%
Bell bird (<u>Anthornis melanura</u>)	10	3	1	6	30%
Chaffinch (<u>Fringilla coelebs</u>)	9	2	0	7	22%
Swan (<u>Cygnus atratus</u>)	2	2	0	0	-
Godwit (<u>Limosa lapponica</u>)	2	2	0	0	-
Red Poll (<u>Carduelis flammea</u>)	5	2	0	3	-
Other species	21	4	0	17	19%
Totals	365	102	7	256	28%