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ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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EDITORIAL NOTE

A fortuitous but significant benefit of our kind of Information Exchange is a periodic overview of an emerging disease problem that is of importance not only to the individual investigators. In this issue the various and detailed contributions on hemorrhagic fever studies provide a collective pattern of what is emerging as perhaps the most pressing global virus disease problem facing our collective field and laboratory research resources.

Recognition, in the last issue, of the recrudescence of yellow fever - definable as one of the hemorrhagic fevers - is now followed by a spontaneous series of reports on other hemorrhagic disease problems of viral etiology.

The emergence of new hemorrhagic fevers in three continents during the past twenty years is perhaps the most ominous basis for anticipating that other arbovirus and rodent associated viral infections can evolve into serious epidemic diseases. Viral hemorrhagic disease in the U.S.S.R. has erupted in newly recognized epidemic manifestations from the Pacific Ocean coast of Asia across the world's largest land mass to the Caspian, Black and Baltic Seas of Europe. Mosquito-borne viral hemorrhagic fevers have appeared and spread in southeast Asia. Argentinian and Bolivian hemorrhagic fevers have become epidemic in continental South America. And other arboviruses have, on occasion, resulted in hemorrhagic infections of man.

Involvement with any one or several of these entities leads to consideration of the common features of all, contrasting the great diversity of viruses which induce infections that result in hemorrhagic complications. Efforts, such as the World Health Organization Regional Seminar on Mosquito-borne Haemorrhagic Fevers in the Southeast Asia and Western Pacific Regions, held in Bangkok in 1964, and the Annual Conference of the Institute for Poliomyelitis and Encephalitis in Moscow emphasize not only the growing magnitude of and concern for these disease problems, but an accumulating productivity of information from detailed study of each.

It is of significance that reports of widely separated efforts should form, in this issue of the Information Exchange, a common ground for inquiry, not so much in regard to the virology and epidemiology of the specific entities as what is not known about the pathogenesis, detection,

treatment and prophylaxis of hemorrhagic disease that results from such a wide variety of etiological viruses. Conceptual questions have begun to develop from a multiplicity of reports. This is the ultimate reward for the continued serious attention the participants give to the contributions they submit to the Arbovirus Information Exchange.

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REPORT OF THE CHAIRMAN OF THE SUBCOMMITTEE
ON ARBOVIRUS INFORMATION EXCHANGE

The Arthropod-borne Catalogue.

Since the report on the Catalogue in the last issue (No. 14) of the Information Exchange, the registrations of 13 "new" viruses have been received, making a total of 204. The registration cards of these new viruses, together with additions to the current information and abstract file, have been issued each three months.

Including the first quarterly issue of this year, 3,889 3" x 5" slips have been distributed, including 2,130 abstracts from Biological Abstracts, 1,546 from Bulletin of Hygiene and Tropical Diseases Bulletin, and 213 personal communications.

As of April 1967, 128 Catalogues including the current information files have been distributed: 53 within continental U.S.A. and 75 to overseas addresses involving 41 different countries.

In the last issue of Info Exchange it was stated that publication of the Catalogue was under consideration. It will be noted from the following report by the chairman of the Publication Committee that publication has been approved and that considerable progress has been made to this end.

It should be clearly understood, however, that the restricted or working Catalogue will continue to be operated as heretofore.

Publication of the Arthropod-borne Virus Catalogue.

Plans for publication of the Catalogue have been crystallized by the ACAV, following the generally favorable response of contributors to the Catalogue. For this purpose the 3-man Subcommittee on Arthropod-Borne Virus Information Exchange has been supplemented by the appointment of a Catalogue Publication Committee, whose members are: Drs. Taylor, Work, Shope, Nathanson (Chairman), Buescher, Casals, Shelokov, and Miss Gladys Sather.

A meeting of this group was held in San Juan, Puerto Rico in November, 1966, and it was agreed to proceed with publication of the Catalogue, which would be essentially a facsimile of the cards in the active Catalogue, as of February 1, 1967. The published version will in no way

supplant the working Catalogue, but will simply make its contents widely available. It is planned to reproduce the Catalogue by photo-offset, which will permit rapid publication and a relatively low price. The published Catalogue will include a preface with historical and explanatory material, but will not include the large 3 x 5 card file which accompanies the working Catalogue. It will be possible to cite the Catalogue as a bibliographic source but this should not interfere with publication in a scientific journal of viruses which have initially appeared in the Catalogue. It is hoped that the published Catalogue will be available in Summer or early Fall.

REPORT FROM DEPARTMENT OF MICROBIOLOGY, JOHN CURTIN SCHOOL OF MEDICAL RESEARCH, AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA, AUSTRALIA

A survey of the incidence of arboviruses in the Territory of Papua and New Guinea was commenced in October 1961. A general surveillance of the Territory has been conducted with the cooperation of the Public Health Department, and intensive investigations have been concentrated in a study area based on Maprik and incorporating approximately fifty miles of the middle reaches of the Sepik River.

Previous communications to the Information Exchange have included summarized results of a comprehensive longitudinal serological survey of native children in the area, and of the immune status of a large sample of wildlife and domestic chickens. These surveys indicated that Group A and B arboviruses were circulating in the area and that activity increased with the proximity of the river and its associated lagoons, channels and swamps. Although conversion rates were fairly high, if the fantastically high mosquito attack rate in the river area is considered it can only be concluded that either the pest mosquito, Mansonia uniformis, plays no part in arbovirus transmission or that the infection rate in these mosquitoes is so low that attempts to isolate viruses from them would be futile. Catching methods which tended to minimize the overwhelming dominance of this mosquito were used more and more exclusively as the investigations proceeded.

No viruses were isolated from about 150,000 mosquitoes, or from small numbers of human pathological material or blood and organs of wildlife collected during the first two and one-half years of the study. Two viruses were isolated from about 70,000 mosquitoes during the following hear; these proved difficult to adapt to suckling mice and have only recently been confirmed.

The situation changed radically in 1966 and from material collected during the first five months of that year 37 viruses have been isolated from mosquitoes, 30 from sera of P.U.O. patients at the hospital for natives at Maprik, and 10 from sera of wild birds. Collections in the study area were terminated at the end of June 1966 and a considerable amount of material collected during this period and also late in 1965 remains to be tested. The number of isolations is hindering rapid characterization, but to date one has been found to belong to Group A and is related to Sindbis virus and four belong to Group B. The latter appear to be more closely related to each other than to known Austral-

asian Group B viruses.

This sudden burst of activity in an area in which arboviruses appeared to be quietly endemic could be a reaction to abnormal climatic factors. Subnormal rainfall in the wet season of November 1964 - April 1965 presaged an unprecedented drought later in the year. This was followed by prolonged heavy rainfall both in the study area and in the main highland catchments of the Sepik River. The ensuing annual flood reached higher levels than previously recorded and the flood plain remained inundated for over three months. Water birds, which had deserted even long-established rookeries during the drought, returned in large numbers. Mosquito numbers on the river were not as high as usual, presumably because of flooding of breeding sites, but there was a significant increase in numbers in the well-drained foothill region. The relative and absolute numbers of mosquitoes of the genus *Ficalbia* increased significantly in both areas, and twelve of the isolates have been from pools of this genus. *Mansonia uniformis*, although not at its usual "haze" level, remained the dominant species on the river, but has not so far yielded a virus.

REPORT FROM DEPARTMENT OF PREVENTIVE MEDICINE VIROLOGY LABORATORY UNIVERSITY OF QUEENSLAND, ST. LUCIA, BRISBANE, AUSTRALIA

A serological survey of pig sera for HI antibodies to a number of the arboviruses occurring in Queensland is being undertaken. Untreated pig sera contained non-specific inhibitors to all the group B viruses tested, and the group A viruses except Sindbis. Acetone extraction of the sera removed all non-specific inhibitors except those to the Group A virus Ross River. The non-specific inhibitor for Ross River virus could be removed by kaolin absorption. Antibodies to Ross River virus and to Murray Valley encephalitis virus have been demonstrated.

Chickens inoculated subcutaneously or intramuscularly with Murray Valley encephalitis virus and Sindbis virus developed a symptomless viraemia that persisted for three to seven days, and produced HI and N antibodies. Inoculation of chickens with Koongal virus did not result in viraemia, nor were detectable levels of antibody produced. (P. D. Spradbrow, Y. S. Chung)

REPORT FROM THE DEPARTMENT OF BACTERIOLOGY UNIVERSITY OF SINGAPORE, SINGAPORE

Studies on Aedes aegypti and A. albopictus:

Studies on the seasonal fluctuations of *Aedes aegypti* and *A. albopictus* mosquitoes are now nearing completion. Adult mosquitoes of each species were collected once weekly from each of three fixed collecting stations in the city area. *A. aegypti* mosquitoes were collected inside houses by aspirator tubes for a period of three hours in the morning at each station. *A. albopictus* mosquitoes were collected from the field, by the human bait method, for one hour during the afternoon at each station. Four persons collected for *A. aegypti* while only two collected for *A. albopictus*. The number of mosquitoes of each species collected during the twelve months (eleven months for *A. albopictus*) of 1966 is shown in Tables I and II. The seasonal fluctuations of the two *Aedes* mosquitoes are shown in the Figure. Both mosquito species have three density peaks. The *A. aegypti* peaks occurred in March, July and October, while the *A. albopictus* occurred in March, June and November. The mosquito population of both species does not seem to fluctuate with the rainfall.

Three viruses have been isolated from *A. albopictus* mosquitoes, and they have been tentatively identified as one dengue type 1 and two dengue type 2 viruses. A dengue type 2 virus has also been isolated from *A. aegypti* mosquitoes.

Dengue Haemorrhagic Fever:

More than 350 cases of haemorrhagic fever have been confirmed in the laboratory by virus isolation or serological tests, during the twelve months of 1966. An increase in HF admissions in the hospitals was first noted in March, and the majority of cases were in the second half of the year. About 50 per cent of the cases were in the age group of 5 to 14 years. A number of deaths have occurred, including both children and adults. Table III shows the results of laboratory studies on 11 fatal cases. During the year, more than 100 viruses were isolated from the acute phase serum of patients. Preliminary testing of a number of these viruses showed the presence of dengue virus types 1, 2 and 3.

(Y. C. Chan and B. C. Ho)

TABLE I. ADULT *AEDES* MOSQUITO SURVEY IN SINGAPORE: JANUARY TO DECEMBER 1966*

SPECIES	STATION	NUMBER OF MOSQUITOES COLLECTED		
		FEMALE	MALE	TOTAL
Aedes aegypti	Dickson Road	475	219	694
	Rayman Avenue	398	176	574
	Henderson Square	433	242	675
	TOTAL:	1,306	637	1,943
Aedes albopictus	Lorong 29	1,872	4,067	5,939
	McNair Road	3,354	3,942	7,296
	College Road	1,097	2,474	3,573
	TOTAL:	6,325	10,483	16,808

* Collection of *Aedes albopictus* began from February.

TABLE II. ADULT AEDES MOSQUITO SURVEY IN SINGAPORE: JANUARY TO DECEMBER 1966

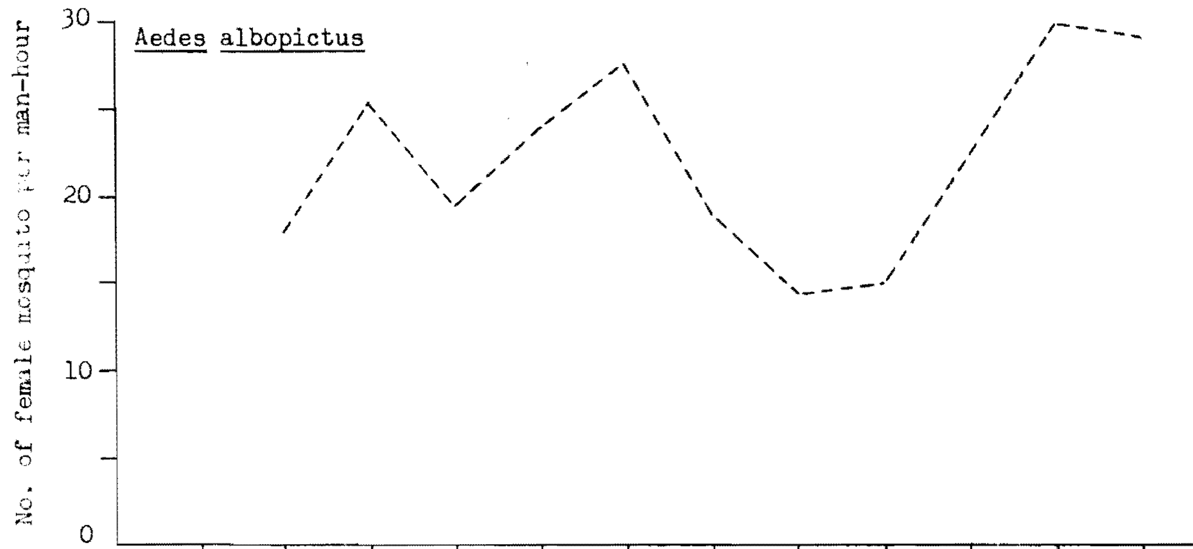
SPECIES	STATION		NUMBER AND STATUS OF FEMALE MOSQUITOES	
			FED/GRAVID	UNFED
Aedes aegypti	Dickson Road		288	187
	Rayman Avenue		260	138
	Henderson Square		287	146
		TOTAL	835	471
Aedes albopictus	Lorong 29		508	1,364
	McNair Road		454	2,900
	College Road		141	956
		TOTAL	1,103	5,220

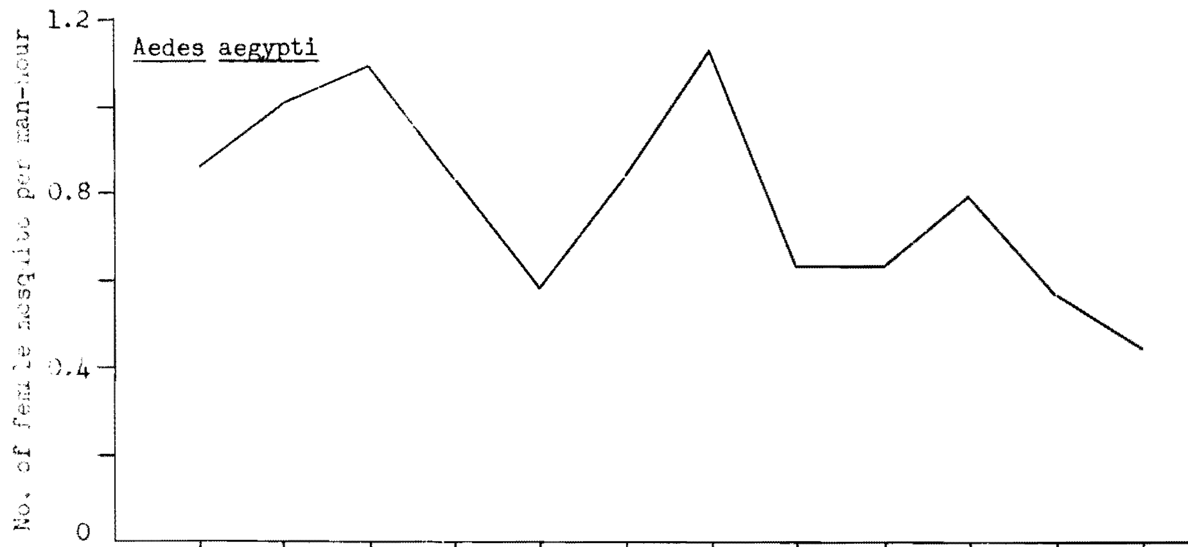
TABLE III. FATAL HAEMORRHAGIC FEVER CASES – 1966

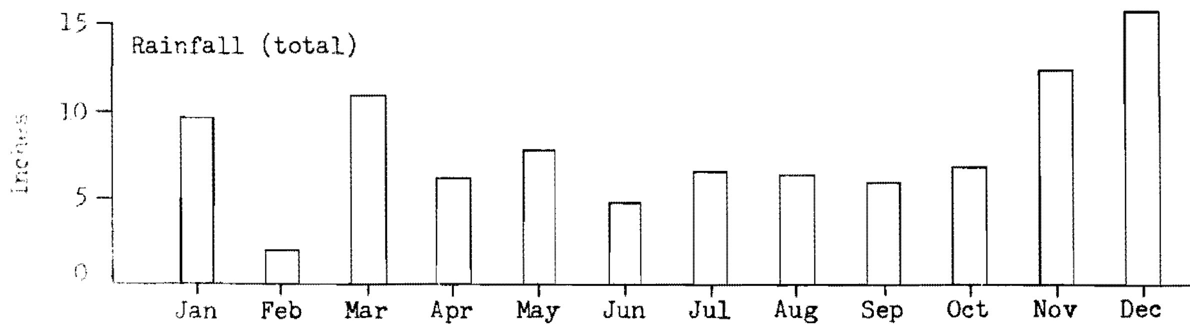
LAB. NO.	SEX	AGE (YRS.)	VIRUS ISOLATION/SOURCE	SERUM/DAY OF ILLNESS	COMPLEMENT-FIXING ANTIBODY TITRE AGAINST				
					JE	D-1	D-2	D-3	D-4
S-114/66	M	9	Dengue-2/ acute serum	AC*/4	<8	<8	<8	<8	<8
GH-12/66	M	9	Dengue-2/ lung						
GH-15/66	M	13		HS†/3	16	128	256	256	256
GH-26/66	M	26		HS/6	128	128	512	256	512
S-782/66	M	4/12	Dengue-3/ acute serum						
S-885/66	F	9	Dengue-2/ acute serum	AS/5 HS/9	32 512	256 2048	256 >4096	128 2048	512 >4096
GH-33/66	F	16		HS/?	>512	>512	>512		
S-897/66	F	7		AS/8	>512		>512		
S-1113/66	F	40		AS/4 HS/5	256 256	>512 >512	>512 >512		
S-1221/66	M	11		AS/? HS/?	8 64	128 128	128 128		
GH-40/66	F	18		HS/?	16	128	128		

* AC = Acute serum; † HS = Post-mortem heart serum.

MONTHLY COLLECTIONS OF ADULT AEDES MOSQUITOES IN SINGAPORE 1966







REPORT FROM VIRUS DIAGNOSTIC LABORATORY, SHIMOGA, MYSORE, INDIA

The epidemic season of Kyasanur Forest Disease started even earlier in 1966 - 1967 than last year. KFD virus isolation from dead monkey tissues was achieved in the month of October for the first time since the beginning of investigations in 1957. (Sridhar Upadhyaya)

REPORT FROM SCHOOL OF TROPICAL MEDICINE, CALCUTTA, INDIA

Haemorrhagic Fever:

Haemorrhagic fever, which broke out in epidemic form in Calcutta during 1963, continued unabated during 1964. In 1965 there were only a few cases. Virological and serological investigations were carried out in collaboration with the Virus Research Centre, Poona. Chikungunya virus was isolated from 64 patients (35 in 1963, 28 in 1964 and one in 1965), and dengue virus (type 2) from three cases (one in 1963, two in 1964). In 1965 three strains of dengue virus (two type 4 and one untyped yet) were isolated from dengue-like fever cases. Chikungunya virus was also isolated from two out of 15 pools of *Aedes aegypti* (total 464) caught in the affected houses. Although dengue virus etiology could be established in many of the HF cases, there were a number of cases of HF from whose blood chikungunya virus was isolated and whose paired samples of sera showed definite rise of antibody again chikungunya.

No case of HF has been reported in 1966.

Serological Survey for Chikungunya Antibody:

In a previous serological survey carried out in 1960, human sera collected from Calcutta were tested for antibodies against Dengue (type 1 and 2), JE, WN, KFD and Sindbis virus. More than 80 per cent of sera had HI antibodies against Group B viruses, but all were negative for Sindbis. Neutralisation tests carried out with dengue, WN and JE antigens indicated that infection by dengue virus was responsible for this antibody against Group B viruses.

In 1964, 494 and in 1965, 539 human sera were collected. These, along with the available 93 sera of 1960, have now been tested against chikungunya antigen by HI tests. 13.6 per cent of 1960 sera, 18.8 per cent of 1964 sera and 32.3 per cent of 1965 sera have been found positive by HI test. This shows that chikungunya or a like virus was active in Calcutta before the outbreak of HF in 1963, although the virus was isolated from Calcutta (and India) for the first time in 1963. The youngest person with positive serum among the 1960 group was 26 years of age, but 20 per cent of individuals of age group 0 - 9 years in 1964 and 11 per cent of that age group in 1965 had their sera positive to chikungunya. (J. K. Sarkar, S. N. Chatterjee and S. K. Chakravarty)

REPORT OF THE VAN HOUWELING LABORATORY FOR MICROBIOLOGICAL RESEARCH, SILLIMAN UNIVERSITY MEDICAL CENTER, DUMAGUETE CITY, PHILIPPINES

Studies on the epidemiology of Philippine Hemorrhagic Fever (dengue-related) in progress in this laboratory include mosquito collections in residences of clinical hemorrhagic fever patients. In order to standardize collections, it was considered necessary to evaluate the effect of the time of day when mosquitoes were collected on the catch obtained, as well as to observe variations in mosquito collections among houses of similar construction and similar habitats, and among consecutive days of collection.

Plan of Study:

Mosquito collections were made from eight houses in a middle-class residential area in Dumaguete City. All houses were of wood or wood and woven bamboo wall construction, unscreened, with closable louvered windows and located on grassed lots with trees and flowering plants. Dogs but no livestock were present on the study premises. The study covered a 10-day period during which the average daily temperature and daily rainfall were as follows:

Date, November 1966:	3	4	5	6	7	8	9	10	11	12
Average daily temperature in °F.:	83.75	80.0	82.0	82.75	82.5	82.5	82.0	83.0	83.5	84.0
Total daily rainfall in inches:	0.08	-	-	0.10	-	1.50	1.80	0.65	-	-

Three rooms in each house (average 3698 cu. ft., range 1807-5516 cu. ft.) were used for mosquito collections. At the time of collections the rooms were completely closed, the floors were covered with newspapers and the atmosphere saturated with Vapona® aerosol. After 10 minutes, the dead mosquitoes were gathered. Day "O" prestudy collections were made on a 2-hour staggered schedule between 5:00 a.m. and 7:00 p.m. in the study homes. Subsequent collections were made at each home at 26-hour intervals from the previous collections except that the 7:00 p.m. collections were followed by 5:00 a.m. visits two days later (34 hours). By using this progressive schedule of collections, the same personnel, trained to be capable observers, were used to collect mosquitoes from each house and at each time within a nine-day period.

Results of the Study:

The mosquitoes collected in the study are shown in Table 1. Nearly all mosquitoes collected were Culex pipiens fatigans, with only a few Aedes aegypti, Aedes albopictus, Anopheles spp. and Culex vishnui complex mosquitoes. The Aedes spp. mosquitoes collected were found to be present in the houses at all times of the day. The analysis of variance (simple and orthogonal) technique was used to test significance of differences in numbers of mosquitoes collected under the several study conditions. The variation in number of mosquitoes collected at different times was significant (F=7.50***, d.f.=7); the variation in number of mosquitoes collected in different houses was significant (F=4.30***, d.f.=7); and the variation in number of mosquitoes collected on different days was not significant (F=1.27 n.s., d.f.=8). The total number of mosquitoes present in the houses was greatest during the hours of highest daylight (7:00 a.m. - 3:00 p.m.). Although the eight premises provided similar general habitats, the mosquito populations varied widely. Mosquito populations in the study houses were uniform over the study period.

Conclusions and Further Studies:

Mosquito collections made in houses between one hour after sunrise and one hour before sunset yielded the most uniform and the largest total catches. Further studies on differences in mosquito populations among premises and on seasonal variations in mosquito populations are in progress.

TABLE I

TOTAL NUMBER OF MOSQUITOS COLLECTED AT EACH HOME
ON EACH DAY, WITH THE TIME OF COLLECTION
(Number of *Aedes* *spp*. Mosquitos in Parentheses)

Home		Day of Collection 1	2	3	4	5	6	7	8	9
H.G.	Time of visit	5 am	7 am	9 am	11 am	1 pm	3 pm	5 pm	7 pm	-
	No. mosquitos	27	100 (1)	104	65	298	120	81	32	
P.U.	Time of visit	7 am	9 am	11 am	1 pm	3 pm	5 pm	7 pm	-	5 am
	No. mosquitos	114	194 (1)	137	89 (1)	159	85 (1)	41		14
G.P.	Time of visit	9 am	11 am	1 pm	3 pm	5 pm	7 pm	-	5 am	7 am
	No. mosquitos	176	175 (1)	132	136 (1)	53 (1)	31		21	233
C.P.	Time of visit	11 am	1 pm	3 pm	5 pm	7 pm	-	5 am	7 am	9 am
	No. mosquitos	238	148 (1)	96	24	28		31	152	146
H.L.	Time of visit	1 pm	3 pm	5 pm	7 pm	-	5 am	7 am	9 am	11 am
	No. mosquitos	57	44	28	23		22	74	82	70
R.P.	Time of visit	3 pm	5 pm	7 pm	-	5 am	7 am	9 am	11 am	1 pm
	No. mosquitos	242	117	46		50	277	275	138	209
L.B.	Time of visit	5 pm	7 pm	-	5 am	7 am	9 am	11 am	1 pm	3 pm
	No. mosquitos	194 (1)	23		3	13 (1)	98	66	62	38 (1)
Z. L.	Time of visit	7 pm	-	5 am	7 am	9 am	11 am	1 pm	3 pm	5 pm
	No. mosquitos	92		19 (1)	131	184	124	189	208 (1)	111 (1)

REPORT FROM VIROLOGY DEPARTMENT, SEATO MEDICAL RESEARCH LABORATORY BANGKOK, THAILAND

Dengue Hemorrhagic Fever:

An outbreak of hemorrhagic fever on the island of Koh Samui in the Gulf of Thailand during the period of July to September 1966 was studied in some detail. The insular location of the outbreak offered an unusual opportunity to study dengue hemorrhagic fever in a small population (approximately 25,000) with relative certainty of identifying all significantly ill patients. A total of 137 patients were studied clinically and virologically; of these, 87 were dengue infections. The cases were classified by the clinical syndrome and type of antibody response.

Table 1 compares the antibody response with clinical syndrome. In the six cases of hemorrhagic fever with shock, 4 had a secondary type antibody response and in 2 cases the type of response could not be determined with certainty. A single fatality due to shock occurred in a child with a secondary type response. None of the severely ill patients had a primary type antibody response.

Twenty-two dengue viruses have been isolated from acute phase sera of these patients. Of these, one is dengue-1, five are dengue-2 and six are dengue-3, the remaining being as yet untyped. It is of interest that three different dengue types were circulating simultaneously in this small outbreak.

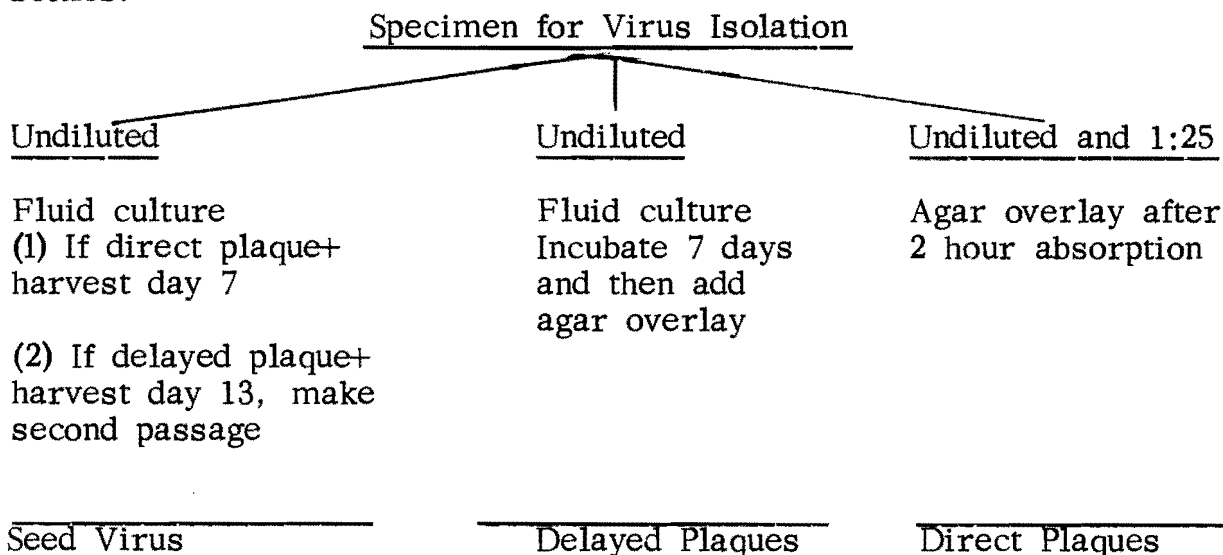
Entomologic studies, including larval collections and biting collections, confirmed the presence of both *Aedes aegypti* and *Aedes albopictus* on the island. Virus isolation attempts yielded four strains of dengue-2 and five untyped viruses from pools of *Ae. aegypti*; and two strains of dengue-2 and two untyped viruses from pools of *Ae. albopictus*.

Dengue Virus Isolation Methods:

Virus isolations from acute phase sera and mosquitoes associated with hemorrhagic fever and dengue-like illnesses have previously been attempted in this laboratory in suckling mice and BS-C-1 cell culture. The BS-C-1 challenge virus resistance technique has been very sensitive for detection of dengue and Japanese encephalitis virus. However, this method has two major disadvantages. First, blind passage is often required with the attendent danger of cross contamination, and second,

an inconveniently large number of tube cultures is required when many specimens must be processed. The knowledge that all arboviruses known to be present in Thailand (i.e. four dengue types, chikungunya, sindbis, tembusu and Japanese encephalitis) produce plaques in LLC-MK_2 cell cultures led to the use of the plaque method for isolation attempts. Preliminary use of this technique for detection of dengue viremia in monkeys led to the observation that delaying application of the agar overlay for seven days after adsorption of the test specimen by maintaining the culture with fluid maintenance medium, increased the sensitivity of this method. Of 32 agents recovered from acute phase human sera, nine were recovered only by the delayed plaque technique (Table 2). The schema for the direct and delayed plaque isolation method is as follows.

Virus isolation system employing LLC-MK_2 cell cultures in 1 oz. R_x bottles.



The plaque isolation method has also been useful for recovery of viruses from mosquitoes (Table 3). The delayed plaque method was employed for isolation attempts from <u>Aedes albopictus</u> collected while biting where holding of the mosquitoes for digestion of blood meals was not possible. Isolation of dengue-2 virus from <u>Aedes aegypti</u> and <u>Ae. albopictus</u> pools by direct and delayed LLC-MK_2 plaques was definitely superior to the use of infant mice (Table 3).

(Philip K. Russell, Douglas J. Gould, Ananda Nisalak, Thomas M. Yuill, Charas Yamarat)

Table 1. Distribution of Cases by Clinical Syndrome and Serologic Response to Dengue

Clinical Syndrome	Dengue HI test Positive			Dengue HI test Negative	Totals
	Primary	Secondary	Un-determined		
HF with shock	0	4	2	0	54
HF without shock	2	24	11	11	
Possible HF	2	14	1	6	83
FUO	5	19	3	23	
Totals	9	61	17	50	137

Table 2. Recovery of Plaque-forming agents from acute phase serum by direct and delayed plaque methods.

	No. Isolations by Indicated Method		
Virus	Direct Only	Delayed Only	Both
Dengue-1	0	0	1
Dengue-2	0	2	3
Dengue-3	0	1	5
Untyped	0	6	14
	0	9	23

Table 3. Virus isolations from mosquitoes from Koh Samui

Pool No.	Species	Plaques in LLCMK-2 cells		Suckling Mice		Virus type
		direct	delayed	Ill	CVR***	
BKM-497	Ae. aegypti	3	nt*	0	12/12	?
BKM-527	" "	3	nt	0	12/12	?
BKM-536	" "	5	nt	11/12**	-	dengue-2
BKM-540	" "	8	nt	0	7/12	dengue-2
BKM-542	" "	20	nt	0	11/12	dengue-2
BKM-545	" "	8	nt	0	0	?
BKM-546	" "	30±	nt	2	8/10	?
BKM-547	" "	30±	nt	6/16	nt	dengue-2
BKM-551	" "	30±	nt	4/16	nt	?
BKM-773	Ae. albo.	0	31	0	11/12	dengue-2
BKM-842	" "	0	35	0	0	dengue-2
BKM-844	" "	18	nt	0	12/12	?
BKM-875	" "	12	nt	0	10/12	?

* nt = not tested
** No. ill or dead/No inoculated
*** CVR - resistant to 100 LD_{50} dengue-2 challenge

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

Virus Isolation of Acute-phase Blood Clots from Hemorrhagic Fever Patients in Manila, Philippines:

An attempt to test the sensitivity of different methods for virus isolation was plotted in this laboratory. Forty-four blood samples were collected from HF patients during their acute phase of illness. Because of the improper storage of the acute sera, blood clots were used in the experiment. After homogenizing with approximately equal volume of 0.5% BA in PBS solution, aliquots of the samples were inoculated into suckling mice (one day old) and MK2 cells respectively. The infected mice were observed for symptoms for 21 days, and the brains of the mice which showed illness were harvested for consecutive passage in suckling mice. The inoculated MK2 cell tubes were checked for CPE under a microscope for seven days, and a blind passage was carried out on the seventh day. As shown in Table 1, highest isolation rate was obtained with the Interference Test Method[1] and with the Plaque System under Noble agar overlay. Observation for CPE in MK2 cells produced the lowest isolation rate.

The virus isolation rate of the 44 blood clots was tabulated according to consecutive days from the onset of illness. Table 2 shows a pattern of increasing isolation rate which starts on the second day, reaches a peak on the third, and decreases on the fourth.

Though this is a very preliminary report, it is interesting to note that we were able to obtain five virus isolates out of 10 blood clots which were collected on the third day after onset of illness (a 50% isolation rate). This is equal to or greater than the virus isolation rate from HF patients using acute-phase sera.[2] The isolates are now undergoing identification procedures, and the paired sera from the HF patients are being titrated.

References: Halstead, Scott B. et al. (1964): Assay of Mouse Adapted Dengue Viruses in Mammalian Cell Cultures By an Interference Method. Proc. Soc. Exp. Biol. and Med. 115: 1062-1068.

Schulze, Irene T. and Schlesinger, Walter (1963): Plaque Assay of Dengue and Other Group B Arthropod-borne Viruses under Methyl Cellulose Overlay Media. Virology 19: 1, January, 1963.

Table 1.

COMPARISON OF VIRUS-ISOLATION OF ACUTE BLOOD CLOTS
FROM HF PATIENTS IN MANILA, PHILIPPINES

	CPE in MK2 Cells: 1st Passage	CPE in MK2 Cells: 2nd Passage	Inoculation in Suckling Mice	Plaquing in* MK2 Cells under Noble Agar Med.	Interference Test in MK2 Cells
POSITIVE RATE	0/44**	2/44	5/44	12/44	14/44
POSITIVE PERCENTAGE	0	4.5	11	27	32

* The MK2 TC fluids of the 2nd passage were used for plaquing and interference test.

** Top number = number positive
Lower number = number tested.

Table 2.

CONSECUTIVE DAYS FROM THE ONSET OF THE ILLNESS
ON WHICH BLOOD SAMPLE WAS TAKEN FROM HF PATIENTS

Day of Blood Collecting	No. of Blood Clots Tested	No. of Virus Isolates	Positive Percentage
1	6	1	17.0
2	12	5	41.6
3	10	5	50.0
4	10	2	20.0
5	3	1	33.3
6	2	1	50.0*
7	1	0	0

* Not conclusive due to the small number of samples.

REPORT FROM DEPARTMENT OF PEDIATRICS,
NATIONAL TAIWAN UNIVERSITY COLLEGE OF MEDICINE,
AND U.S. NAVAL MEDICAL RESEARCH UNIT NO. 2, TAIPEI, TAIWAN,
AND DEPARTMENT OF PREVENTIVE MEDICINE,
UNIVERSITY OF WASHINGTON, SEATTLE, WASHINGTON

Inactivated Japanese Encephalitis (JE) Vaccine from Cell Cultures of Hamster Diploid Cell Strains:

The potency of Inactivated Japanese Encephalitis Tissue Culture Vaccine was compared with Japanese Mouse Brain Vaccine (MBV) by testing in mouse and monkey.

The result of mouse protection test indicated that MBV protected better against intracerebral challenge of Nakayama strain but not against a recently isolated Taiwan strain (HVI), while the Inactivated Tissue Culture Vaccine (ITCV) protected the animal better against HVI strain than the Nakayama strain. In monkey protection test, ITCV produced high antibody titer (1:160 by HAI test) in all monkeys while MBV produced detectable antibody in only four out of six vaccinated monkeys and only one of which had a titer of more than 1:40. All of the monkey sera were tested with the antigen prepared from the Nakayama strain which was homologous to MBV seed but heterologous to ITCV. After these animals were challenged intranasally with HVI strain (heterologous to both types of vaccine seeds) five out of six control monkeys and four out of six monkeys vaccinated with MBV died of typical encephalitis, but all of six monkeys which received ITCV survived the challenge after various grades of encephalitis symptoms. Two out of six monkeys previously infected with JE virus still having a high titer of antibody also developed encephalitic symptoms after the same type of challenge.

The vaccine had been tested in vitro and in animals including suckling and adult mice, young hamsters and guinea pigs for the presence of exogenous microorganisms, carcinogenic effects (also tested with viable cell suspension up to 10^6 per 0.1 ml.), weight loss or any other untoward effects. The results were usually negative.

(George C. Y. Lee and E. Russell Alexander)

REPORT FROM 406th MEDICAL LABORATORY, DEPARTMENT OF VIRAL AND RICKETTSIAL DISEASES, U.S. ARMY, JAPAN

Dr. Wiebenga, a member of National Institute of Allergy and Infectious Diseases, N.I.H., arrived in August to assume responsibility for the department and expand previous studies of hemorrhagic fever in Korea.

From October through January team members from this department and from the Department of Entomology, under LTC V. Tipton, MSC, USA, visited Korea. This team was based at the 121st Evacuation Hospital, Ascom, but they also collaborated with personnel of the ROKA 7th Evacuation Hospital, south of Oii jon bu. We were also fortunate to obtain the part-time services of Edward Tyson, Ph.D, who was in Korea on other business.

Previous five-year data led us to expect about 10 cases in the U.S. Forces and about 100 in the ROKA Forces. Thus the total of 36 cases in U.S. Forces during calendar year 1966 was unexpected. Of this total, 31 occurred between 7 October and 31 December. Three additional cases were hospitalized during the first week of January, bringing the total number of cases to 34 during the current epidemic season. The mortality rate in this group was 5.5%. As of 31 December 1966, 82 cases in ROKA Forces were reported by Colonel Hyon, Commanding Officer of the 7th Evacuation Hospital.

Since previous epidemiological studies remain inconclusive in the absence of etiologically related antigen or antibody, we concentrated our efforts on the collection of specimens from acute cases.

Specimens were collected from about 65 acute cases. About 40% of these were inoculated immediately into cell cultures, suckling mice, or hamsters. About 20% were inoculated into all these systems. All specimens were shell frozen, stored and transported at -60° C. or lower. Laboratory study of this material is in progress, and there are plans to increase laboratory facilities here and in Korea, to better handle the isolation material and to expand our studies next season.

REPORT FROM DEPARTMENT OF MICROBIOLOGY, KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

Survey of Anti-Arboviral Antibodies in Sera of Residents of Indonesia:

Three hundred sera were taken from residents in the Lampung State, southern Sumatra, Indonesia, during the period from August to September, 1965. HI antibodies against dengue types 1 and 2 (D1, D2), Japanese encephalitis (JE), and yellow fever (YF) viruses were measured by the microtiter technique (Takatsy-Sever). The data so far obtained indicate: (1) D1 is widely distributed, while D2 is rare; (2) JE is thought to be endemic, though in minority; and (3) Anti-YF antibodies are detected, because of the comparatively low titers, however, the positive YF reactions are presumably due to crossing with other arboviruses, especially dengue group viruses. Further investigations along the same line are being undertaken.

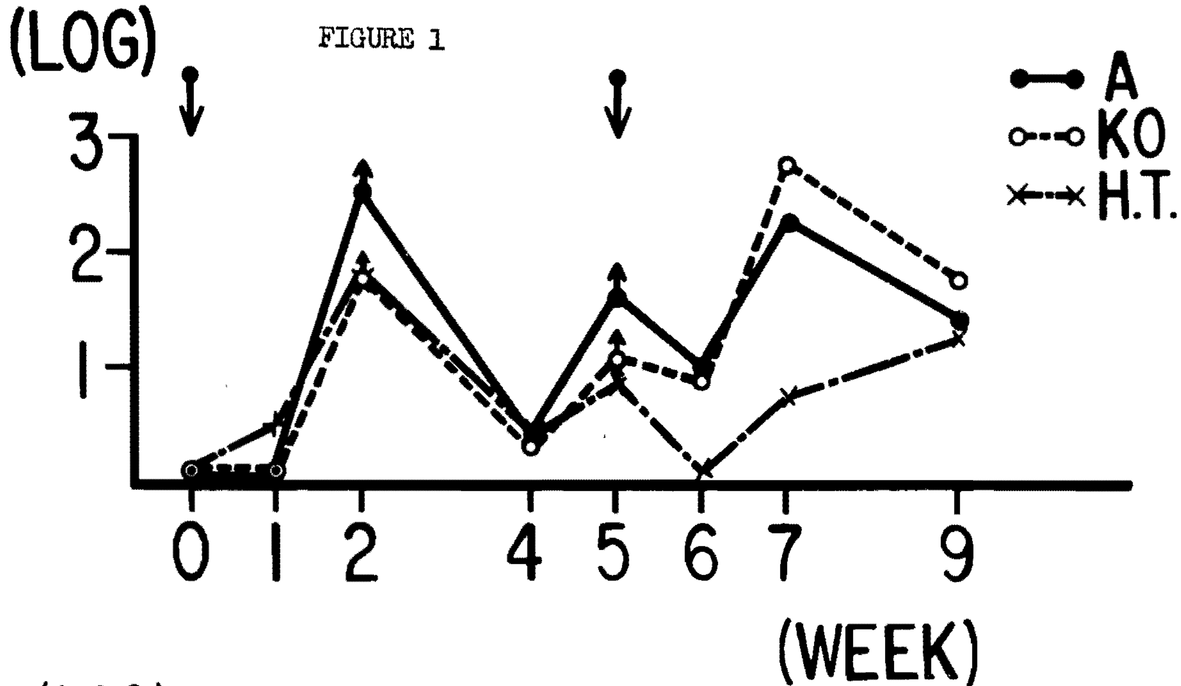
Immunogenic Effect of a Tissue-Cultured (Human-Attenuated) Type 1 Dengue Virus Strain:

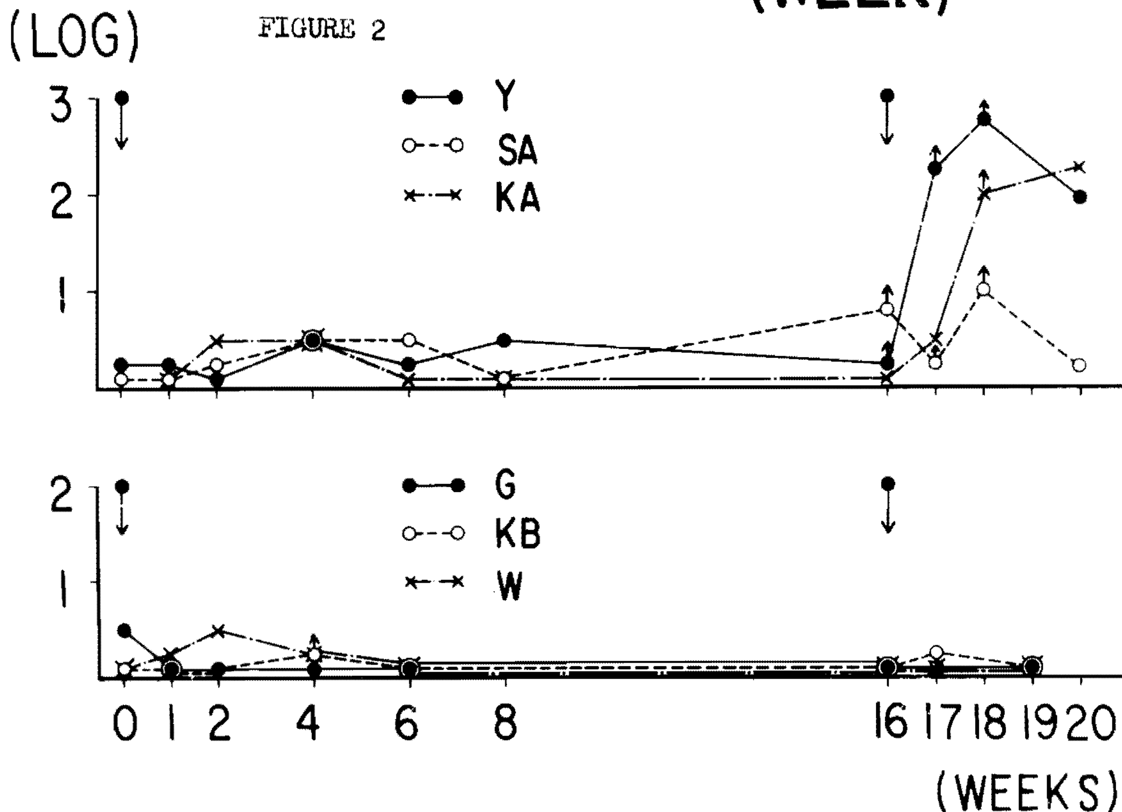
Two series of experiments were conducted as an extension of the previously reported (Refer to this Infoexchange, No. 13, p. 69, 1966). Type 1 Mochizuki strain virus, passed in mouse brains for more than 160 generations and subsequently in *M. fuscatus* monkey kidney cell cultures for more than forty generations, was inoculated into human adults intracutaneously. Conditions of the inoculation were similar to those described previously. At intervals after the inoculations, sera were taken and anti-dengue (type 1) neutralizing indices (NI's) were measured. Examples of the results obtained are summarized in Figures 1 and 2.

In Figure 1, three adults (A, KO and HT) were inoculated with living virus. In Figure 2, three adults (Y, SA and KA) were inoculated with living virus, and the other three (G, KB and W) with formalinized virus. Ordinates indicate NI's expressed by logarithmic number, and abscissae indicate period of observation in weeks.

After a single inoculation of the living virus, neutralizing antibodies were detectable in some of the sera. The NI's could rise more rapidly and reach higher levels after the second or third inoculation. No such immunizing effects were evident in the subjects inoculated with the formalinized virus.

All the individuals were examined carefully by physicians, but no abnormal signs such as temperature rise, malaise, headache, changes in total and differential leucocyte counts, etc., were noted. It was presumed from the data that the modified Mochizuki strain virus remained attenuated for humans even after being passed in tissue cultures and still caused a "symptomless infection" which was accompanied by production of neutralizing antibodies.





REPORT FROM THE CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH AND THE ROCKEFELLER FOUNDATION ARBOVIRUS STUDY UNIT, BERKELEY, CALIFORNIA

There were nine confirmed and one presumptive cases of Western encephalitis in man in California in 1966. The first case occurred in San Joaquin County, with onset July 19th. This was a confirmed case in a 2-1/2 year old boy. There was another confirmed case in the same county with onset August 14th. There were two cases in July, five confirmed and one presumptive in August and two in September. There were three cases in Sacramento County and one each in Merced, Santa Clara, Shasta and Stanislaus Counties. The presumptive case was from Tulare County. This case was a six-weeks-old child with spinal fluid changes consistent with the diagnosis of encephalitis and a single blood specimen with a CF titer of 1:32 with the WE antigen. There were no fatalities.

There were 21 confirmed cases of Western encephalitis in horses in California during 1966. There were four cases in July, 15 in August, one in September and one in October. The disease was most active in Sacramento, Fresno and Kern Counties. There were cases in Butte, Humboldt, Imperial, Sonoma and Tulare Counties. Western encephalitis virus was isolated from the brain tissue of a tree squirrel, *Sciurus griseus*, found sick on August 2nd in Chico, Butte County.

There were four confirmed cases of St. Louis encephalitis. These occurred in Marin, Sacramento, San Mateo and Yolo Counties between August 7th and September 15th. There were four presumptive cases of St. Louis encephalitis where paired bloods showed only a two-fold rise in the CF titer with St. Louis virus antigen. These occurred during the same time period as the confirmed cases. One of these was presumed to have been infected in Dallas, Texas.

There were seven cases of Colorado tick fever, two with onset in April, two in June and three in July. Four were contracted from tick bite outside of California. Two were exposed in Plumas County and one in Modoc County. The Colorado tick fever virus was isolated from the blood specimen in each instance.

A new virus, called the Mono virus, has been isolated from Ar 861, a pool of 10 adult Argas cooleyi ticks collected from California gull nests on a bird island in Mono Lake, Mono County, California, May 18, 1966. Cross-immunity studies show a relationship to Hughes virus.

Some new information has been obtained about Modoc virus in the course of a study of a new strain of this virus isolated in 1966. This strain of Modoc virus M2249-M2253 was isolated from a pool of submaxillary salivary glands and lungs of five Peromyscus maniculatus mice trapped May 18, 1966 near Lee Vining, Mono County, California. Two hamsters were inoculated with the original specimen by the IM route. When these were killed four months later, the salivary glands and kidneys were tested for virus. The tissues were rinsed with saline to remove the blood. One of the hamsters had active virus in the kidney tissue, killing all of eight infant mice inoculated with the 10^{-1} suspension with an AST of 10 days. This hamster kidney specimen was subinoculated into two hamsters by the IM route to study excretion of the virus in the urine. One of the hamsters inoculated with this specimen has shown a chronic viuria. Urine specimens were tested without dilution by addition of antibiotics and IP inoculation into infant mice. Urine specimens taken on the 13th, 21st and 68th days have been positive for virus. A neutralization test of the virus isolated from the 68-day urine specimen was positive for Modoc virus.

The study of an attenuated WE live virus vaccine for horses was continued in 1966. A total of 1232 horses were vaccinated. There were no illnesses attributable to the vaccine.

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

Virus Isolation Studies:

From early in 1961 through June of 1966 we have isolated a total of three arboviruses from numerous mosquito pools tested. All three of these agents were identified as being SLE-like viruses, one isolate being obtained in October 1962 and the other two in September of 1964. (These isolations have been described in previous editions of the Information Exchange). Mosquitoes collected from early in August, 1966 through September 1966 have shown a much increased rate of arbovirus activity. From these mosquitoes we have isolated 12 suspected arbovirus agents, all from pools of Culex quinquefasciatus. Five of the isolated have been tentatively identified as SLE-like viruses on the basis of HI tests using HA antigens prepared by sucrose-acetone extraction of infected suckling mouse brain. Suspected isolates have been

obtained from mosquitoes collected at three different locations in and around Tucson. Identification of the other viruses is currently in progress.

Sentinel Flocks:

The increase in SLE virus activity in the Tucson area suggested by the virus isolations described above has also been observed in sentinel chick flocks. The following table indicates the results so far obtained:

Location of Sentinel Flock	Approximate Bleeding Dates		
	May 1966	Late August 1966	Early November 1966
I. City Jail Farm, Tucson	0/50*	12/42	7/31
II. University Cattle Farm, Tucson	0/15	0/14	7/12
III. Boyce Thompson Arboretum, Superior	0/15	0/15	0/15

* Number of SLE HI titers 20≥ / Total number of sera tested

Neutralization tests were performed in suckling mice with 11 of 12 HI positive sera from the August bleeding of Group I chickens. Six were definitely positive, five probably positive but should be repeated and one equivocal and also in need of repeating. None of the other HI positive sera have yet been tested for NT antibody. Two of our isolates have been from mosquitoes collected in the chicken house of the City Jail Farm, location I, while no viruses have been isolated from mosquitoes collected at the University Cattle Farm, location II. Location III is not in the Tucson area but some 100 miles or so north of the city.

WE virus activity has been much less pronounced. One chicken from the August bleeding of the City Jail Farm chickens had positive HI titers in November as did one from the University Cattle Farm, but it has not yet been determined whether or not these results are specific or due to an inhibitor of some kind. The indications of limited WE virus activity was not too surprising as we continued to find a very low level of *Culex tarsalis* activity. During 1966, of some 21,000-plus mosquitoes

captured, less than 600 were Culex tarsalis.

There was one serologically confirmed human case of St. Louis encephalitis reported in Tucson this fall. We are currently testing human serum samples collected in October and November of 1966 to see if there is any indication of increased cirus activity in the human population of this area.

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

IN COLLABORATION WITH THE COLORADO AND THE TEXAS STATE DEPARTMENTS OF PUBLIC HEALTH

Colorado:

During the summer of 1966 in Colorado there were six confirmed cases of WE and six of SLE giving a rate of 0.3 per 100,000 for each of these diseases. Three of the six cases of WE were in children less than one year of age, giving a rate of seven per 100,000. The three cases of WE in infants occurred in Mesa County, an area in which no cases of WE had been reported since 1958.

Virus infection rates among C. tarsalis collected in Colorado during 1966 were very low, as were the C. tarsalis light trap and shed trap population indices. Sixty-seven pools of C. tarsalis, 25 specimens per pool, were tested for virus and only single isolations of WE virus and Turlock virus were obtained. The seasonal WE infection rate, and also the Turlock infection rate, was 0.6 per 1,000 mosquitoes when calculated by the method of Chiang & Reeves. The sentinel chicken flock seasonal transmission indices also were indicative of medium levels of virus activity during the year. The 53 chickens in the two sentinel flocks were 21 percent WE and 12 percent SLE positive for antibody in HAI tests.

Texas:

In the Hale County area 35 human suspect cases of encephalitis were investigated during the summer of 1966. Of this group 11 cases were confirmed as WE and three as SLE. This gives a rate of 30 per 100,000 for WE and eight per 100,000 for SLE. Seven of the 11 cases

of WE were in children less than one year of age, giving a rate of 708 per 100,000.

The virus infection rates among C. tarsalis collected in Hale County were again high during 1966. A total of 667 pools, 25 C. tarsalis per pool, was tested and preliminary results indicate 217 WE virus isolates, eight Turlock isolates, and five SLE isolates. At the peak of the WE virus amplification cycle (July 24-30) the C. tarsalis virus infection rate was 47/1000. The seasonal WE virus infection rate was 15/1000, whereas the rates were approximately 0.4/1000 for each of the other two viruses.

Twenty-two percent of 269 nestling house sparrows tested for virus during the summer were positive for WE virus, as were 12 percent of 71 nestling blackbirds. At the peak of the WE virus amplification cycle (July 24-30) 10 of 11 nestling house sparrows sampled were positive for WE virus. The 12 flocks of sentinel chickens maintained in the same area were 85 percent positive for WE antibody and 58 percent positive for SLE antibody on the basis of HAI tests of the mid-October blood specimens.

A portable six-volt mechanical aspirator for collecting adult mosquitoes was developed and tested during 1966. The unit was field-tested in Colorado, Texas and Hawaii and found to be especially useful for collecting mosquitoes from natural resting places. Some of the advantages of the collecting unit include its relatively low cost, its economical operating expense, its direct collecting action that eliminates the need to transfer specimens, its collection of undamaged specimens, its quiet operation, its ease of operation, and the speed with which large numbers of resting or flying mosquitoes can be collected.

REPORT FROM LABORATORY SECTION, STATE HEALTH DEPARTMENT, AUSTIN, TEXAS

The first case of St. Louis encephalitis at Dallas was confirmed August 9 by LBCF significant titer increase. One hundred and thirty-five cases in the 1966 outbreak subsequently were confirmed by the LBCF or HI micro technics. In retrospect, it appeared that several cases had occurred in July, but the maximal number occurred in mid-August, the majority of cases observed among the adult population. Although the

cases were first thought to be concentrated along the Trinity River, it soon became evident that cases were occurring in many areas of Dallas and Dallas County. At least 19 deaths were attributed to SLE. The epidemic was aborted, it was suggested, by aerial spraying of Malathion August 19-27.

Eighty-three additional suspect SLE cases were confirmed in Corpus Christi, Fort Worth and other areas. The SLE virus was recovered from a fatal case at Brownwood. Fourteen suspect WE cases mostly from the irrigated areas of West Texas were confirmed. Several additional SLE and a few WE cases were tabulated as "presumptive" or "inconclusive".

Thirteen equine infections were confirmed and several others were "presumptive" or "incomplete" in WE tests. These infections were widely scattered over the state.

A total of 29,763 mosquitoes, primarily *Culex quinquefasciatus* or *Culex tarsalis* were pooled and tested for arboviruses. Twenty-one virus isolates were obtained, and were identified as 14 Hart Park, five SLE, and two WE. Pools of *Culex quinquefasciatus* collected in Ft. Worth yielded 13 of the 14 Hart Park and four of the SLE isolates. Hart Park isolates were obtained from mosquitoes collected in May (1), June (7), and July (5). The four SLE and WE viruses were obtained from mosquitoes collected in July and August. A single pool of *Culex melanoconion* sp. collected by Fort Sam Houston entomology group on the Wildlife Refuge area near Sinton showed presence of WE virus.

Eight arbovirus isolates obtained locally in the Houston City Public Health Laboratory were referred to us for identification. Seven of these were characterized as strains of Hart Park virus and one as a member of the CEV complex. The Hart Park isolates originated from pools of *Culex quinquefasciatus* collected in June, July and August. The CEV virus was isolated from a mixed pool of *Aedes atlanticus* and *Aedes infermatus*. Complement fixation tests with five other members of this group indicates that this possibly is a new member of the group. The following table demonstrated antigenic differences among six of the strains by "cross block" LBCF tests.

Complement Fixation Tests with Members of CEV Complex

Antigen	Anti-sera					
	CEV (LaX)	41079	Trivittatus	CEV (BFS 283)	46713	20230
CEV (LaX)[1]	1:256	1:32	1:4	1:32	1:32	1:32
41079[2]	1:128	1:64	1:8	1:32	1:32	1:64
Trivittatus[3]	1:64	1:8	1:64	1:8	1:16	1:8
CEV (BFS 283)[4]	1:128	1:32	1:8	1:64	1:32	1:32
46713[5]	1:64	1:16	1:16	1:16	1:128	1:16
20230[6]	1:64	1:64	1:8	1:32	1:32	1:64

(1) Obtained from CDC, Atlanta, in 1965
(2) Isolated from pool of Psorophora confinnis, San Benito, Texas in 1966
(3) Obtained from Hammon & Sather in 1961
(4) Obtained from American Type Culture Collection in 1962
(5) Isolated from pool of Aedes atlanticus and Aedes infermatus, Houston, Texas in 1966
(6) Isolated from pool of Anopheles pseudopunctipennis, San Angelo, Texas in 1958

The relationship between CEV (LaX) and the other strains is more apparent than real since these mice received ten rather than the customary five doses of vaccine. Isolates 41079 (San Benito, 1966) and 20230 (San Angelo, 1958) appear to be closely related, if not identical.

(J. Feild, T. S. Sullivan, T. Guedea, and J. V. Irons)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL SCHOOL, DALLAS, TEXAS

When studies of experimental arbovirus infections in bats, some of which were discussed in a previous Exchange (Number 10, October, 1964) had provided evidence that these animals could serve as effective reservoir hosts for these agents, a program designed to obtain field evidence that bats could be involved in the epidemiology of certain arbovirus diseases was initiated. It seemed that efforts in this direction would be best spent in a place where such a disease occurs frequently in epidemic proportions. Since epidemics of JBE occur yearly in certain parts of Japan it seemed likely that the levels of virus circulating in nature in vertebrate hosts responsible for the survival of the agent during interepidemic periods would be higher there than in an area such as the United States, where years may elapse between major outbreaks. Thus a cooperative field study between this department and the Department of Hygiene, University of Tokyo in Japan was initiated in the summer of 1963. Collection of bats, harvest of tissues and assay of a portion of the specimens obtained was under the direction of Dr. Teiji Miura.

The first bats were netted in the summer of 1963 and collections were continued at intervals throughout the three main areas of Japan (Kyushu, Honshu and Hokkaido) into December, 1965. A total of almost 2000 bats were obtained and, with few exceptions, significant numbers of animals were netted during each of the four seasons of the year in all three areas so that the year-round persistence of arbovirus infection in these animals could be determined. Specimens obtained from bats included blood, brown fat, brain, kidney, spleen and salivary glands for virus assay and plasma for antibody determinations. Most of the tissues for virus assay were shipped to Dallas along with a small proportion of the plasmas obtained. Dr. Miura accepted the responsibility of testing larger numbers of bats for serological evidence of JBE virus infection and is also assaying bloods from certain groups of bats for the presence of this agent.

Although the battery of tests required in the assay of such a large number of specimens are far from complete, some evidence of natural JBE virus infection in bat populations in the Kyushu and Tokyo areas has been obtained. Antibodies reactive with JBE virus in neutralization tests have been detected in a number of bat plasmas and a number of viral agents have been isolated from blood specimens. We are

particularly interested in viruses isolated from bats netted during the late fall, winter and early spring months when surveys by other investigators have shown that JBE virus is not detectable in the mosquito population or in known reservoir hosts such as birds and swine. Four of the viral agents isolated from bats netted in Tokyo have been tentatively identified as JBE virus and two of these are from animals captured in October. A number of viral agents, not yet characterized, were isolated from bats netted during the winter months and in early spring. Should any of these agents prove to be JBE virus some insight into the mechanism through which this virus persists in the Tokyo area between epidemics may be gained.

An epidemic of St. Louis encephalitis (SLE) in Houston, Texas in the summer of 1964 encouraged the initiation of field studies in south Texas. Efforts to find colonies of bats in the Houston metropolitan area where the center of the epidemic was located were unsuccessful. A source of bats was found, however, in Angleton, Brazoria County, approximately 40 miles south of Houston, a distance well within the possible nightly flight range of the species of bats (*Tadarida b. mexicana*) collected from the area. The first collection of bats was made on August 26, 1964 which subsequently proved to be during the week when the epidemic reached a peak with regard to number of cases. In order to determine if SLE virus could be detected in the bat population subsequent to the epidemic period and at a time when the agent is no longer demonstrable in the vector or known reservoir hosts, additional groups of bats were netted in September, October, November and December of 1964. Collections were resumed in April of 1965 and continued at approximately monthly intervals through January 1966 to determine if SLE virus infection could be detected in bats in this area during what proved to be a non-epidemic year. A strain of SLE virus was isolated from the blood of one of the 137 bats comprising the collection made on August 26, 1964. A second strain of SLE virus was obtained from the blood of an animal captured November 11, 1964, approximately two months after the last isolation of SLE virus from mosquitoes collected in the area. This would indicate that SLE virus was persisting in the bat population at a time when the agent could not be demonstrated in the vector. Virus isolates, tentatively identified as strains of SLE virus have been obtained from the pooled spleens of four bats netted in April 1965 and from blood specimens from three bats collected in November 1965. Should these agents and other as yet uncharacterized isolates obtained from bats netted in December 1965 and in January 1966 prove to be strains of SLE virus there would be little doubt that populations of *Tadarida b. mexicana* in this area of Texas could serve as effective reservoir hosts in which active SLE virus infection can be

demonstrated during every season of the year.

In the summer of 1966 an outbreak of SLE occurred in Dallas, Texas. As soon as this epidemic became apparent, a program was launched to locate sources of bats in the Dallas area. Although the response of the people to radio, television and newspaper please for information concerning bats was excellent, the information provided was poor. Investigations of locations where large numbers of bats had reportedly been sighted flying about during the early evening hours convinced us that residents in the area were mistaking chimney swifts for bats. A few solitary bats were brought to the laboratory by individuals, but no virus could be detected in tissues from any of these animals.

Thus, we were unable to find sufficient numbers of bats in Dallas or environs at that time to enable us to determine if these animals were harboring SLE virus. This laboratory was able, however, to cooperate with the United States Public Health Service in testing tissues from fatal human cases for the presence of SLE virus. This agent was isolated from the brain tissue of seven of the 10 fatal cases from which specimens were obtained.

A second outbreak of SLE in Texas during the summer of 1966 occurred in Corpus Christi. With the cooperation of Mr. Tex Villarreal of the Corpus Christi-Nueces County Health Department, we were fortunate in locating a bat roost in downtown Corpus Christi. Bats were found roosting along rafters and beams in the ceilings of warehouses in the dock area of the city. The first collection was obtained September 11, 1966 and continued at approximately 14-day intervals through November 1966. Collections are now being made in Corpus Christi at approximately monthly intervals. One agent, isolated from the blood of a bat in the September 11, 1966 collection, has already been positively identified as SLE virus. Other isolates from bats collected in Corpus Christi are being characterized. (S. Edward Sulkin and Rae Allen)

REPORT FROM THE ROCKY MOUNTAIN LABORATORY, HAMILTON, MONTANA

Studies on the Ecology of the Encephalitis Viruses:

During the summer of 1966 we attempted to isolate Western Encephalitis from frogs and snakes by intensively sampling certain areas in Montana. Generally, these were relatively undisturbed areas of short

grass prairie with numerous potholes and prairie sloughs. All had a history of moderate to heavy WE occurrence, and both biting arthropod and herptile populations were high.

Herptiles were collected alive and returned to the laboratory, where the frogs were heart-bled out. Snakes were bled by clipping the tail, tagged, and stored for repeat bleedings. Tissues, including brain, liver, spleen and kidneys, were removed from certain herptiles and processed for virus isolation. Sera were stored for later antibody studies.

Bloods were generally diluted 1:10 with 25% normal rabbit serum saline with 250 units of penicillin and 250 µg. of streptomycin added per ml. Tissues were ground in TenBroeck grinders in roughly 1:10 dilutions of 25% NRS saline with p and s added. Processed bloods and tissues were inoculated intracerebrally into wet chicks (less than 24 hours old) in 0.02 ml. amounts. Two chicks were inoculated with each specimen. Chicks were held and observed over a 14-day period. First-day deaths were discounted. Deaths beyond day three were passed into three-day-old suckling mice by grinding chick brains in 1.5 cc. 25% NRS saline with p and s added. Mice received 0.02 ml. of homogenate i.c. The results of this study are shown in Table I.

In order to insure that the herptile populations sampled were not atypical, we collaborated with Mr. Jeffrey Black, a graduate student in herpetology from the University of Montana who was building a state-wide collection of herptiles. He kindly made available for testing live specimens from widely separated areas of Montana. These were treated as previously described. Results of this work are shown in Table 2.

It will be seen that, from 532 specimens which included 193 rattlesnakes, 59 garter snakes, four bullsnakes, two blue racers, 239 leopard frogs, 10 chorus frogs, five toads, and 10 western spotted frogs, no isolations of any organism pathogenic to suckling mice on passage from chicks were made during this test. (Gordon M. Clark)

Table 1.

Virus Isolation Attempts in Chicks

With Herptile Bloods and Tissues - 1966

Date	Species	Number of Individuals	Source	Material	Isolation	Mouse Passage
5	<u>Crotalus viridis</u>	51	Hamilton	Blood	-	
5	"	5	"	"	+	-
5-19-65*	"	25	"	"	-	
5-19-65*	"	2	"	"	+	-
7-19	"	1	Shonkin	"	+	-
7-20	"	9	E. Fork Bitterroot	"	-	
7-20	"	2	"	"	+	-
7-21	"	11	"	"	-	
10-4	"	22	"	"	-	
10-18	"	18	"	"	-	
1965*	"	20	Havre	"	-	
	"	17	Thomas [1]	"	-	
5	<u>Thamnophis</u> sp.	4	Highwood	"	-	
7-20	"	2	"	"	-	
6-1	"	16	Willow Creek	"	-	
10-18	second bleeding	16	Willow Creek	"	-	
7-18		22	Thomas [1]	liver	-	
7-19	<u>Lampropeltis getulus</u>	2	Highwood	blood	-	
6-19		2	"	"	-	
6	<u>Coluber constrictor</u>	1	Hamilton	"	+	-
6	"	1	"	"	-	
5-31	<u>Rana pipiens</u>	24	Shonkin	blood	-	
6-16	"	10	"	"	-	
6-16	"	40	Highwood	"	-	
6-16	"	3	Highwood	"	+	-
7-20	"	6	"	"	-	
7-20	"	13	Shonkin	"	-	
8-4	"	23	"	"	-	
8-4	"	2	"	"	+	-
8-12	"	17	"	"	-	
8-12	"	24	Willow Creek	"	-	
8-12	"	1	"	"	+	-

Date	Species	Number of Individuals	Source	Material	Isolation Mouse Passage
8-1	*Rana pipiens*	26	Willow Creek	blood	-
8-1	"	6	Shonkin	"	-
8-1	"	as above	"	spleen, liver kidney	-
8-1	"	20	"	"	-
6-16	*Pseudacris clarkii*	10	Highwood	blood	-
6-9-65*	*Bufo borealis*	5	Victor	"	-

* Bloods from 1965 stored at -70F for one year.

1 Snakes donated by Dr. Leo Thomas.

Table 1.

Virus Isolation Attempts in Chicks With

Miscellaneous Herptile Bloods*- 1966

Date	Species		Number of Individuals	Source	Isolation	Mouse Passage
6-29	Thamnophis radix haydeni	286	1	Mud Lake Refuge	-	
6-4	"	262	1	Fort Benton	-	
8-5	"	381	9	10 mi. W. Albion	-	
6-8	"	411	1	Musselshell River	-	
8-5	"	382	1	6 1/2 mi. W. of Albion	-	
[illegible]	"	405	1	Moon Creek, Miles City	-	
6-7	Thamnophis elegans vagrans		1	43 mi. E. of Hamilton	-	
6-5	Rana pipiens		2	1 mi. E. of Whitehall	-	
7-4	"	308	1	25 mi. SW of Choteau	+	-
7	"	282	1	30 mi. SE of Circle	-	
8-8	"	408	1	11 mi. NW Forsythe	+	-
8-4	"	378	1	3 mi. W of Broadus	-	
6-27	"	273	1	3 1/2 mi. S Sidney	-	
6-5	Rana pretiosa	239	1	S. Fork Smith River	-	
6-5	"	241	2	22 mi. E. Townsend	-	
6	"	249	3	30 mi E. Hamilton	-	
7-26	"	363	3	Red Rock Lake	-	
-	"	416	1	N. of Helena	-	
6-5	Bufo boreas	237	3	23 mi. E. Townsend	-	
7	"	371	1	Missoula	-	
7-26	"	362	5	Odel Creek	-	
7-25	"	360	1	5 mi. N. Divide	-	
6-4	"	367	3	Fort Benton	-	
6	"	364	2	Red Rock Lake	-	

* Bloods diluted 1:10 with 25% normal rabbit serum saline

Ecology of Mosquito-borne Encephalitis Viruses:

Investigations of potential overwinter mechanisms of WE and SLE viruses in northwestern United States are being conducted at a study area near Vale, in eastern Oregon.

A. Studies of *Culex tarsalis* during spring:

In 1965 overwintered *C. tarsalis* were observed from the time of emergence from hibernation in March until occurrence of reproduction in late spring. Relatively large populations were found concentrated at isolated groves - "oases" - in the midst of generally arid territory. Blood feeding increased as the season progressed, and incidence of parity, based on ovarian tracheation, also increased accordingly. By the time of appearance of the new brood of adults in late May, 85% of female *C. tarsalis* gave evidence of past or current blood feeding. Dr. C. H. Tempelis of the University of California, Berkeley, conducted precipitin tests on mosquito blood meals. Of 286 specimens, 94% had fed on birds and 6% on mammals (principally bovine). Of the bird feedings, 42% were on passeriforms, 14% on columbiforms, 19% on strigiforms, and 25% on undetermined birds. Such host preference and the distribution and feeding activity of the vector indicate that *C. tarsalis* is potentially able to support virus cycling in May. The findings also suggest small isolated groves as possible sites of origin on yearly virus cycles in this area.

In 1966 marking techniques were used to determine whether mosquitoes found in such localities constitute a stable resident population. *C. tarsalis* were collected during spring in baited traps, and the engorged specimens were marked with fluorescent powder and released. All mosquitoes of each subsequent collection were examined for marking. Of 184 marked specimens released, four were recaptured at the point of original release. Of the four, two were engorged and two contained neither blood nor eggs.

B. Virus activity:

During the active mosquito season of 1965, one of 15 sentinel chickens developed HI antibody to WE, none to SLE. There were four isolations of WE virus and none of SLE from 1070 *C. tarsalis* collected during June, July and August. In 1966, however, there was activity of both viruses as shown in Table 1. There was a qualitative and quantitative difference in the WE/SLE at the three study sites.

Table 1. Virus Activity at Vale, Oregon in 1966

	Sentinel Conversions WE	SLE	Virus Isolations* WE	SLE	?	No. Mosq. Tested
Site 1, in a cultivated area	3/3	0/3	15	1	3	2505
Site 2, an "oasis" in an unirrigated area	3/3	0/3	2	2		902
Site 3, a second oasis	0/4/	0/4	0	0		282
Totals	6/10	0/10	17	3	3	3689

* Identity is based on incubation period in mice.
/ As of August 6, when rates at Site 1 and 2 were 3/3 and 1/3 respectively.

During the springs of 1965 and 1966, before the appearance of appreciable numbers of a new generation of adults, 627 and 327 C. tarsalis respectively, were collected and tested for virus with negative results. This brings the total of mosquitoes collected in the springs of years with subsequent virus activity to 3,355. No evidence of virus infection was found in these mosquitoes. An additional 725 C. tarsalis, taken from hibernation sites during winter, did not contain virus.

(William A. Rush)

Laboratory Studies on Hughes Virus:

A number of attempts to produce viremias in various conventional laboratory animals have been unsuccessful, except in chicks, where a mild viremia results after subcutaneous injection of .05 ml. of stock virus. But here viremias were not sufficiently high and did not occur consistently enough to infect ticks (Ornithodoros denmarki) fed on these animals.

Ticks were successfully infected, however, by inducing them to feed

on a pool of virus-laden blood through fresh mouse skin stretched over a glass cylinder. At various intervals after feeding, two ticks were selected at random, triturated in diluent and intracerebrally inoculated into 3-4-day-old mice. Two ticks fed similarly on uninfected blood were ground at each interval as controls (Table 1). In one experiment virus survived for the 29 days that ticks were available for test. The titer was generally low, but increased significantly after the ticks had molted. In a second experiment the virus survived for 189 days at which time the supply of ticks was exhausted. During this time the ticks had been refed several times and, before its end, all surviving ticks had reached the adult stage. Therefore, it appears that Hughes virus can be transstadially transmitted through several nymphal molts to the adult stage. (C. Clifford)

Table 1

Survival of Hughes virus in artificially fed *Ornithodoros denmarki*

Expt. 1 - *O. denmarki* - 3rd nymphs

Titer of suspension used to feed ticks (log suckling mouse i.c. LD_{50}/03 ml) 5.75		1	3	5	7	9	11	14	16	19	22	26	29
	[Days]	1	3	5	7	9	11	14	16	19	22	26	29
				Ticks Molting									
	[Titer]	1.75	1.15	>1	2.46	1.47	4.58	4.50	1.75	2.15	-	-	2.70

Expt. 2 - O. denmarki - 3rd nymphs*

Titer of suspension used to feed ticks (log suckling mouse i.c. LD_{50}/0.03 ml) about 5													
	[Days]	1	3	5	7	9	12	15	18	21	26	29	34
					Ticks Molting								
	[Titer]	2.32	2.0	2.58	1.5	1.45	-	-	-	>1	-	1.87	1.76
	[Days]	39	43**	47	50	55	61	65	69	74	78	81**	82**
	[Titer]	>1	1.85	2.70	2.40	1.70	3.15	4.40	2.75	3.23	no data	-	3.32
	[Days]	85	91	97	113	120**	131	144	155	182**	189		
	[Titer]	2.50	-	2.75	3.50	>1	2.50	3.50	2.50	1.60	3.50		

* Ticks were 3rd stage nymphs when the experiment started, but molted after blood meals. After 2 feedings almost all the ticks had become adults.

**Ticks all refed on chick.

Invertebrate Tissue Culture

Multiplication of two strains of CTF virus in primary tissue cultures of metamorphosing nymphal *Dermacentor andersoni* viscera was shown. A post-inoculation latent period of 6-10 days was followed by a 4-1/2 log increase of extracellular virus at 4-5 weeks. Virus was recovered in diminishing quantities for as long as 159 days in medium and 166 days in triturated tissues. Certain characteristics of the Florio-2 strain remained unchanged after 98 and 124 days *in vitro*. Virus titers in whole nymphs infected by feeding remained relatively constant from drop-off to molting, but titers in cultures prepared from their tissues increased by approximately five logs two weeks after explantation. No differences between virus-infected and uninfected cultures were seen. Explants of both produced fibroblast-like (FBL) cellular outgrowths within hours of explantation, initially probably from hemocytic migration. Within four days mitotic activity was seen which continued for at least two months. Explants were viable for as long as 152 days, but peak activity occurred 4-6 weeks after explantation. Nymphs fed on hamsters produced larger and longer-lived outgrowths *in vitro* than those fed on rabbits, but calf serum was superior to hamster or rabbit serum as a component of culture medium. Further experiments to determine the relative specificity and sensitivity of the CTF virus *D. andersoni* system are planned.

Cultures of the insect-cell line derived by Grace from *Antheraea eucalypti* were adapted to medium supplemented with fetal bovine serum, egg ultrafiltrate and bovine serum albumin instead of insect hemolymph. Growth of this subline equals or surpasses that of the parent line in medium supplemented with insect hemolymph: its log phase of growth is longer and total cell number achieved is higher. Thus, for the first time, a line of insect cells is available whose use is not restricted by difficult-to-obtain insect hemolymph. Studies of the susceptibility of these cells to infection with arboviruses are under way. (C. E. Yunker)

REPORT OF ANIMAL HEALTH DIVISION, NATIONAL ANIMAL DISEASE LABORATORY, AMES, IOWA

Insect collections from the vesicular stomatitis area were insignificant during 1965. They consisted of 170 insects in 15 pools, all of which were negative. Mosquito collections were made at Ames during 1965. Three isolations were made from 997 mosquitoes. One, a hemagglu-

tinating virus from Culex tarsalis, was made from insects collected during September, during which time clinical encephalitis appeared in horses about 10 miles down river. Two nonhemagglutinating viruses were isolated; one was from Culiseta inornata and one from Culex pipiens and closely related species. These isolates have not been further studied.

A single case of New Jersey-type vesicular stomatitis was diagnosed in Texas during January 1966 followed in April by a reappearance and spread northward. The 1966 New Jersey-type outbreak involved mainly Texas, New Mexico and Colorado.

Of about 600 premises in which clinical vesicular stomatitis was diagnosed in horses or cattle during 1966, 12 were Indiana type; the others were New Jersey type plus some sera which gave cross-type reactions. Two-thirds of the New Jersey type cases occurred in Texas.

The New Jersey type outbreak involved the same area as the Indiana type had involved during 1964 and 1965. There was no known overlap of Indiana VS infected area in 1965 over the area involved during 1964. No human cases were called to our attention during 1966.

Neutralizing antibodies were present in three of 13 swine submitted from four Texas farms on which cattle had shown lesions, but no clinical cases had been observed in swine.

Identification and virus isolation work is progressing on insects collected in Colorado, New Mexico and Texas during 1966.

REPORT FROM THE DEPARTMENTS OF VETERINARY SCIENCE, ENTOMOLOGY AND PREVENTIVE MEDICINE, UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

Six suckling mouse lethal agents were isolated from Wisconsin arthropods in 1966. Fifteen were isolated in 1965 and 13 in 1964. Collection technics employed in 1965 and 1966 were comparable (DeFoliart, G.R. and Morris, C.D., 1967: A dry-cie-baited trap for the field storage of haematophagous Diptera in Wisconsin during 1965. J. Med. Ent. In press). Seasonal and geographical trends in arthropod populations are emerging (DeFoliart, G.R., Rao, M.R. and Morris, C.D.,

1967: The seasonal succession of blood sucking Diptera in Wisconsin during 1965, J. Med. Ent. (in press). The unseasonably long and cold spring of 1966 delayed the emergence and appreciably reduced the populations of some but not all species. It may have contributed to the reduction in number of isolations.

Only one clinical case of Western encephalitis was observed. This was a horse in Dane County that developed a specific increase in CF and neutralizing antibody. Eight cases of California encephalitis, all in children hospitalized for encephalitis, were demonstrated on the basis of specific increase in HI and CF titers to La Cross Strain. Six additional cases have not yet been confirmed. On the basis of the development of antibody in sentinel rabbits, a tree canopy appears to be essential for transmission of California virus. No evidence was obtained of the presence of Eastern or St. Louis encephalitis in man or livestock species. (Robert P. Hanson, Gene DeFoliart, Wayne Thompson).

BLOODSUCKING DIPTERA FROM WISCONSIN THAT WERE CAPTURED AND POOLED BETWEEN APRIL 10 AND SEPTEMBER 20, 1966 FOR ARBOVIRUS ISOLATION ATTEMPTS

Species or Categories	Number Pooled	No. of Pools Processed	No. of Isolates	Years of Previous Isolations
CULICIDAE				
Aedes canadensis	209	11	.	.
Aedes cinereus	151	10	.	.
Aedes communis group	1,731	82	1	64
Aedes excrucians	89	.	.	.
Aedes punctor	381	2	.	.
Aedes spp.	3,877	110	.	.
Aedes stimulans group	570	23	1	65
Aedes trivittatus	4,533	161	1	64, 65
Aedes triseriatus	.	3	.	64 65
Aedes vexans	14,631	374	1	64, 65
Anopheles punctipennis	10	.	.	.
Anopheles walkeri	5	1	.	.
Culex pipiens and/or restuans	77	16	1	.
Culex restuans	60	2	.	64
Culex salinarius	56	4	.	64
Culex spp.	439	12	.	.
Culiseta morsitans	30	2	.	.
Culiseta inornata	304	14	.	.
Mansonia pertubans	7,689	180	1	.
Psorophora ferox	10	1	.	.
Total	34,852	1,008	6	(13) 64, (9) 65
CERATOPOGONIDAE				
Culicoides obsoletus	102	.	.	64
Total	102	.	.	(1) 64
SIMMULUDAE				
Cnephia mutata	5,490	59	.	.
Cnephia taeniatifrons	142	9	.	.
Prosimulium fuscum group	1,672	30	.	.
Simulium johannseni	8,437	93	.	.
Simulium meridionale	285	6	.	65
Simulium venustum group	10,508	124	.	.
Simulium vittatum	496	17		
Total	27,030	338	0	(1) 65

Species or Categories	Number Pooled	No. of Pools Processed	No. of Isolates	Years of Previous Isolations
TABANIDAE				
Chrysops aberrans	9	.	.	.
Chrysops callida	685	37	.	.
Chrysops carbonarius	10	1	.	.
Chrysops cincticornis	50	4	.	65
Chrysops cuclux	33	3		
Chrysops excitans	287	21	.	.
Chrysops frigidus	341	17	.	.
Chrysops indus	551	34	.	.
Chrysops mitis	77	6	.	.
Chrysops montanus	134	7	.	.
Chrysops niger	158	12	.	.
Chrysops sackeni	100	8	.	.
Chrysops striatus	192	4	.	.
Chrysops weidmanni	10	.	.	.
Hybomitra offinis	16	.	.	.
Hybomitra epistates	956	97	.	.
Hybomitra h. hinei	366	26	.	.
Hybomitra illota	1,251	80	.	.
Hybomitra lasiophtalma	4,590	461	.	65
Hybomitra nuda	1,123	115	.	.
Hybomitra trepida	76	9	.	.
Hybomitra trispila sodalis	319	9	.	.
Hybomitra typhus	4,335	292	.	.
Hybomitra zonalis	263	46	.	.
Tabanus lineola	1,126	83	.	.
Tabanus marginalis	4	.	.	.
Tabanus quinquevittatus	15	.	.	.
Tabanus similis	639	52	.	.
Tabanus sparus	626	34	.	.
Tabanus trimaculatus	288	54	.	.
Total	18,630	1,512	0	(2) 65
CHLOROPIDAE				
Hippelates sp.	.	.	.	. 64
Total	0	0	0	(1) 64
ACARINA				
Dermacentor variabilis	499	38	.	.
Ixodes cookei	2	2	.	.
Ixodes marxi	1	1	.	.
Haemaphysalis leporis-palustris	33	6	.	.
Total	535	47	.	.
GRAND TOTAL	81,149	2,905	6	(13) 64 (15) 65

REPORT FROM DEPARTMENT OF MEDICAL BACTERIOLOGY, UNIVERSITY OF ALBERTA, EDMONTON, AND CANADA AGRICULTURE RESEARCH STATION, LETHBRIDGE, ALBERTA

Results from Specimens Collected in 1965:

When the last report was forwarded (September 1, 1966) only a number of the brains, received from horses that died during the epizootic of 1965, had been processed and investigated for the presence of arboviruses. We report here the results of the work completed on all the brains received. Isolation of virus was attempted by inoculation of each brain specimen into chick embryo cell cultures and into the amniotic cavity of eight-day-old embryonated hens' eggs, as well as by intracerebral and intraperitoneal injection of one-two -day-old suckling swiss white mice. The procedures used with chick embryo cell cultures and embryonated hens' eggs were those described by Lennette and Schmidt (Diagnostic Procedures for Viral and Rickettsial Diseases, Third Edition, 1964). The techniques used for isolation attempts in mice have already been described in the September 1, 1966 report. WE virus was isolated from five brains out of 35 tested. The virus was recovered in all the host systems used. Identification of the isolates was performed in suckling mice by intraperitoneal injection. From 30 brains no agent was recovered. During the investigation it was learned that a number of the brains had been kept frozen on dry ice, without adequate sealing, before shipment to the Laboratory Branch of the Provincial Veterinary Services from which we received the specimens.

Blood sera were received from three horses that later died and from the brains of which WE virus was recovered. Complement-fixing antibodies to WE, EE, and SLE viruses were investigated with the three sera, but NT antibodies only with two sera. Antibodies were detected only with WE virus (see Table 1). Of the five positive horses, three came from Edmonton, where WE virus activity was demonstrated for the first time. HO-3 came from a pet shop, but HO-1 and HO-4 both were the only horses on the farm and had never moved from Edmonton. HO-1 was a draft horse and HO-4 was the children's pet.

Results from Specimens Collected in 1966:

In contrast to the epizootic of equine encephalomyelitis of 1965, no outbreaks of sleeping sickness have been reported in 1966, only few sporadic cases. The Virus Laboratory received paired sera from three horses and no positive findings were recorded. A brain of a

dead horse was also received, and no viral agent was isolated.

No human cases suspected to be ill with WE encephalitis were reported to the Laboratory. The investigation of arbovirus incidence in Alberta was continued by serological studies of animal sera and attempts at isolation of viruses from arthropods and other specimens.

Chicken Sera from the Irrigated Areas of Lethbridge:

Four sentinel flocks of 20 roosters each, hatched in the middle of May, were set out at the middle of June into the same areas investigated during the previous two years. Capons were not used in 1966, since differences in antibody response were not detected in 1965 when a comparative study was conducted with roosters simultaneously exposed in the same field locations. The birds were tagged, bled before exposure in the field and bled at approximately two-week intervals thereafter. From June 13 until November 1, 709 chicken sera were collected aseptically by veni-puncture and tested by hemagglutination-inhibition (HAI) test for evidence of infection with WE, EE and SLE virus. The methods used have been described in the 14th issue of the Arthropod-borne Virus Information Exchange.

This year no positive findings were recorded in Luco and McAdoo areas where conversion from negativity to positivity of WE infection was demonstrated in 90 percent of the birds in 1965. Only one chicken in the Nilsson area and four chickens in the eight-mile Lake area showed WE antibodies, whereas 33 percent of the birds in the Nilsson area and 100 percent in the eight-mile Lake area were positive in 1965 (See Table 2).

Domestic Rabbits:

Fifty-eight domestic rabbits were bled and set out on June 14 into the same areas of the sentinel chicken flocks. The rabbits were bled only once after exposure in the field, October 21st. However, at that time only 26 of the original 58 were still alive, the remainder having been killed by wild animals. Of the 26 animal sera tested none was positive for EEE and SLE virus, and only one showed antibodies to WE virus. The positive animal was located in the Luco farm.

Wild Animals:

Twenty-three wild animals were caught in the irrigated areas of the

south of Alberta. Of these 13 were gophers and 10 wild birds (four red-wing black birds, two black birds, one sandpiper, one duck, one curlew, one pelican). From each animal the following specimens were received: blood serum, blood clot, lung-kidneys pools and brain. The blood sera have all been tested for the presence of antibodies to WE, EEE and SLE virus. None of them was positive. The viscera blood clots and brains are being tested in suckling mice and final results are not yet available.

Mosquitoes

One hundred and seventy pools, of 10-30 mosquitoes, have been collected in mammalian burrows of the areas under investigation in the same manner as described in the last report. In 1966 again the same three species as in 1965 were found to be present in the burrows: C. inornata, C. tarsalis and A. earlei. Collections were made from July 1 until October 30 (See Table 3). No collections were made by the sweep-net method in the field. All pools are being investigated for arboviruses in suckling mice. No results are available as yet.

(Dr. Odosca Morgante, J. A. Shemanchuk)

Table 1. Virus Isolated from the Brain of 5 Horses that Died of Encephalitis in 1965

Animal No. & Age	Location	Onset of Disease	Date of Death	Specimen	Date Rec'd.	Virus Isolated	Antibody Titre To WE Virus (CF(1:)	
								Log LD 50 NT Index
HO-1 10 years	Edmonton	VIII:14	VIII:17	Brain	VIII:18	WE		
				Blood	VIII:16		16	1.7
HO-2 6 years	Red Deer	VIII:16	VIII:17	Brain	VIII:19	WE		
HO-3 Age Unknown	Edmonton	VIII:17	VIII:19	Brain	VIII:19	WE		
				Blood	VIII:18		32	2.0
HO-4 Age Unknown	Edmonton	VIII:21	VIII:23	Brain	VIII:30	WE		
				Blood	VIII:23		32	NSQ
HO-5 24 years	Rimbey	IX:8	IX:9	Brain	IX:10	WE		

NSQ = Not Sufficient Quantity.

Table 2. HAI Antibodies to WE Virus in Chicken Sera from Sentinel Flocks of Roosters, Located in Four Areas of Lehtbridge.

	Luco	McAdoo	Nilsson	8-Mile Lake
June 13	0/20	0/19	0/20	0/19
July 18	0/20	0/20	0/20	0/19
August 5	0/19	0/20	0/20	0/20
August 15	0/19	0/20	0/19	2/20
August 29	0/19	0/20	0/20	2/20
September 12	0/19	0/20	1/20	4/20
September 26	0/19	0/20	1/20	4/20
October 10	0/19	0/20	1/20	4/20
November 1	0/19	0/19	1/20	4/20

Table 3. Number of Mosquito Pools Collected from Mammalian Burrows in Alberta in 1966

	Number of Pools Collected								
	July		August		September		October		
	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-30	Total
C. inornata	6	3	0	9	56	53	0	0	127
C. tarsalis	0	3	0	3	14	6	0	0	26
A. earlei	0	0	0	1	0	1	0	15	17
Total	6	6	0	13	70	60	0	15	170

REPORT FROM THE WESTERN COLLEGE OF VETERINARY MEDICINE,
UNIVERSITY OF SASKATCHEWAN, SASKATOON,
THE CANADA AGRICULTURE RESEARCH STATION, SASKATOON,
DEPARTMENT OF BIOLOGY, UNIVERSITY OF SASKATCHEWAN,
SASKATOON, AND THE PROVINCIAL LABORATORY,
DEPARTMENT OF PUBLIC HEALTH, REGINA, SASKATCHEWAN

Western Encephalitis in Saskatchewan in 1966:

Only two human and eight horse cases of Western encephalitis (WE) were reported in Saskatchewan during the summer of 1966. This low incidence of the disease in man and horses was matched by low infection rates in six sentinel chicken flocks, by low infection rates in English sparrows, low Culex tarsalis and Culiseta inornata mosquito populations and apparently also by low infection rates in these mosquitoes.

In the six sentinel chicken flocks only 16 out of 144 birds, or 11.1%, acquired infections, most during the latter half of August and early in September, and one half (8) of the infected birds were in the Estevan flock located in the endemic, southeast corner of the Province. In the previous non-epidemic year (1964) of our study, 11.8% of the birds in the six flocks acquired infections, but in the epidemic year (1965) 70.8 % became infected and these in the period from the middle of July to the third week of August.

To date, 287 blood samples from 13 wild bird species collected during the summer of 1966 have yielded 18 isolations of WE virus, all from nestling birds; but only seven from English sparrows (187 tested), five from Barn swallows, two from Magpies, two from Loggerhead shrike, one from a Starling and one from a Franklin's gull. In the epidemic year 1965, 15 isolations of WE virus were obtained from 118 bird bloods tested, of which 38 were from English sparrows and 12 out of the 15 isolations were from the English sparrows.

In 1966, the total mosquito population of the season, as gauged by our six light traps, was second only to that of the epidemic year 1965, but the catches of Culex tarsalis and Culiseta inornata were the second lowest we have taken in a season in the five years of the study, being lower only in 1964, the other interepidemic year. A high proportion of WE virus isolations from mosquito species other than C. tarsalis, particularly during a non-epidemic year, was noted in the previous issue, and it was again apparent in 1966. From 584 mosquito species pools preserved for examination for WE virus in 1966, 18 isolations

of WE virus have been obtained. Only three of the 18 isolations have been from *C. tarsalis*, three have been from *C. inornata*, five from *Aedes vexans* and the remaining seven from five others, namely *A. flavescens*, *A. spencerii*, *A. dorsalis*, *A. campestris* and *Anopheles earlei*.

In the spring of 1966 it was evident that a reservoir of WE virus was available to mosquitoes. The first isolation of WE virus of the season was obtained from the blood of a *Peromyscus* mouse caught and bled on March 29. From April 7 to May 6, in one area near Saskatoon, the virus was obtained from the bloods of 21 garter snakes (*Thamnophis* spp.) out of 188 bled and in the period from May 6-31 from 31 frogs (*Rana pipiens*) out of 137 bled. Thereafter the virus appears to have circulated, as indicated above, in a bird - mosquito - bird cycle but with fewer domestic birds and English sparrows involved and the mosquitoes predominantly species other than *C. tarsalis*.

Repeated bleedings of captive, naturally-infected garter snakes (*Thamnophis* spp.) and frogs (*Rana pipiens*) have shown that these animals can carry inapparent WE infections that are undetectable by available methods as viremia or neutralizing antibodies in the blood. Viremia in these recurs most often in response to a lowering of the environmental temperature, but occasionally also in animals held continuously at room temperatures. Out of 30 garter snakes that gave no evidence of infection when first tested, 25 (or 83.3%) were subsequently found to be infected, either by isolation of virus from their blood or by detection of SN antibodies, or both. Viremia also recurred in infected female garter snakes when they gave birth to their young. It is noteworthy that in Saskatchewan, garter snakes are born early in August when populations of *C. tarsalis* and *C. inornata* are at or near their seasonal peaks in abundance.

The Provincial Public Health Laboratories in Regina are now supporting work being carried on by Connaught Medical Research Laboratories in Toronto, directed toward the production of a vaccine against the virus of WE. (J. McLintock, A. N. Burton, H. E. Robertson, and J. G. Rempel).

REPORT FROM THE HOSPITAL FOR SICK CHILDREN TORONTO, CANADA

Powassan virus isolations were achieved from three of 60 pools of Ixodes cookei ticks removed from 286 groundhogs (Marmota monax) which were collected some 200 miles north of Toronto between May 5 and September 5, 1966. Virus yields per pool of one to 11 ticks ranged from $10^{2.5}$ to $10^{6.0}$ TCD_{50} for primary swine kidney tissue cultures, and positive pools were collected on June 24, July 15 and August 10. Powassan neutralizing antibodies were detected by mouse inoculation tests in 143 of 362 animals including 127 of 286 groundhogs, 14 of 45 red squirrels (Tamiasciurus hudsonicus) and two of 31 other forest mammals. The monthly prevalence of antibody in the current season's groundhogs increased from zero to 25 percent with the progression of summer, but in older animals the incidence remained between 38 and 62 percent throughout the season. These results substantiate earlier findings which pointed towards the maintenance of Powassan virus in nature by a cycle involving groundhogs plus squirrels as reservoirs, with ticks as vectors, from which human infections occurred tangentially.

REPORT OF THE ENCEPHALITIS FIELD STATION, LAKEVILLE HOSPITAL, MIDDLEBORO, MASSACHUSETTS

The first case of Eastern Encephalitis (EE) in horses in Massachusetts since 1956 was reported this year. A three-year-old unvaccinated colt in the town of Middleboro exhibited symptoms similar to those of EE on August 5. The horse was destroyed two days later, and the entire head was submitted to the Livestock Disease Control Center. A portion of the brain was sent to the Virus Section of the State Diagnostic Laboratory on August 11. Confirmation of the isolation of EE virus was received at the Field Station from the Virus Section on August 17. Although other horses in the area were unvaccinated, no additional cases were found.

Despite this isolation, very little arbovirus activity was noted in southeastern Massachusetts. No virus was isolated from 202 spring migratory birds (April 25 - June 1) in chick embryo tissue cultures (CETC). Since 1961, 1600 spring migrants have been tested and found negative

for EE and WE virus.

No virus was isolated from 1064 summer resident and fall migratory birds (June 6 - November 1).

No coastal survey of immature fall migrating birds was undertaken in 1966. During 1964 and 1965, immature birds of species known to breed only to the north or west of Massachusetts were sampled at Duxbury Beach. All samples were tested for EE and WE virus (CETC) and neutralizing antibody (Plaque Reduction). During 1964, blood samples were taken from 263 of the 585 captures and in 1965 from 330 of 886 captures. WE antibody was recovered from a single sample - a white-crowned sparrow captured and bled on October 17, 1965. Zonotrichia l. leucophrys breeds from the Hudson Bay region east to the Atlantic coast.

No virus was isolated from 559 blood samples, 305 brain specimens, or 123 pooled or single visceral organ samples taken from 576 mammals during 1966, nor from 36 reptile bloods. The majority of these samples were tested in CETC, although some were inoculated into suckling mice. Plaque reduction neutralization tests are currently being attempted with these serum samples. Results of the 1965 and 1966 antibody surveillance in non-avian vertebrates completed to date are shown in Table 1.

The 1966 arthropods are presently being tested for virus in suckling mice. Plaque reduction tests for neutralizing antibody in sentinel chickens and rabbits have not been completed. Results will be reported at a later date.

Table 1. Serological test results on non-avian samples collected in Massachusetts during 1965 and 1966 (number positive/number tested).

	1965				1966		
	Virus*	Antibody**			Virus	Antibody	
	EE & WE	EE	WE	POW***	EE & WE	EE	WE
Opossum					0/2	0/1	0/1
Shrew, Short-tailed	0/4	0/3	0/3	0/3			
Bat, Little Brown					0/3	0/3	0/3
Raccoon	0/3	0/3	0/3	1/3	0/20		
Otter, River					0/1		
Weasel, Long-tailed					0/5	0/3	0/3
Ermine					0/1		
Mink					0/16		
Skunk, Striped	0/12	0/12	0/12	10/12	0/13	0/5	0/5
Fox, Red	0/1	0/1	0/1	0/1	0/26		
Fox, Gray					0/1	0/1	0/1
Woodchuck	0/4	0/4	0/4	2/4	0/1		
Squirrel, Red	0/7	0/5	0/5	0/4	0/10	0/4	0/4
Squirrel, Gray	0/88	0/56	0/56	0/35	0/10	0/7	0/8
Chipmunk, Eastern	0/14	0/14	0/14	0/13	0/47	0/35	0/36
Beaver					0/1		
Mouse, White-footed	0/155	0/106	0/106	1/44	0/135	0/129	0/126
Vole, Gapper's Redback	0/14	0/14	0/14	0/13	0/9	0/8	0/9
Vole, Meadow	0/29	0/29	0/29		0/2	0/2	0/2
Muskrat					0/155		
Mouse, House	0/21	0/21	0/21	0/1	0/2	0/2	0/2
Rat, Norway	0/14	0/13	0/13	0/13	0/39	0/37	0/37
Mouse, Meadow Jumping	0/6	0/4	0/4	0/6			
Cottontail, Eastern	0/53	1/44	0/44	0/24	0/8	0/7	0/7
Rabbit, Domestic					0/2	0/2	0/1
Hare, Snowshoe					0/5	0/5	0/3
Deer, White-tailed					0/45		
TOTAL MAMMALS	0/425	1/329	0/329	14/176	0/559	0/251	0/248
Turtle, Snapping	0/2	0/2	0/2		0/1	0/1	0/1
Turtle, Painted	0/22	0/72	0/72		0/7	0/6	0/6
Turtle, Spotted	0/18	0/20	0/20		0/11	0/11	0/11
Turtle, Wood					0/1	0/1	0/1
Turtle, Box	0/3	0/3	0/3		0/7	0/7	0/7
Turtle, Musk	0/4	0/4	0/4		0/1	0/1	0/1
Snake, Black Racer	0/2	0/2	0/2		0/1	0/1	0/1
Snake, Northern Water	0/3	0/3	0/3		0/1	0/1	0/1
Snake, Eastern Garter	0/7	0/7	0/7		0/6	0/6	0/6
TOTAL REPTILES	0/61	0/113	0/113		0/36	0/35	0/35

* CETC or Suckling Mice

** PR

*** Neutralization in suckling mice by the Yale Arbovirus Research Unit (Dr. W. C. Downs).

REPORT FROM YALE ARBOVIRUS RESEARCH UNIT, NEW HAVEN, CONNECTICUT

Filtration of Arboviruses Through Millipore(R) Membranes:

During current attempts to fit the arboviruses within a universal system of classification, it has become clearly apparent that there are severe deficiencies in the knowledge of basic physical, structural and biochemical properties of these agents, on which the proposed systems are mainly based.

While size of a virus is not one of the properties whose knowledge is needed for its classification in one of the schemes proposed (Tournier and Lwoff: Symposia, 9th International Congress for Microbiology, 1966, 417-422), it nevertheless seems logical that size should have a connection with at least an element used in that classification; markedly dissimilar sizes within a collection of viruses may reflect differences in the numbers of capsomers as well as in the triangulation numbers of agents with cubic symmetry. Besides, heterogeneity in size may correspond with heterogeneity in other properties.

The behavior of arboviruses upon filtration through Millipore(R) membranes is being investigated in this laboratory. Since factors other than size may affect the filtrability of viruses through such membranes (one such factor may be the recently reported presence of detergents in the membranes) and since the number of types in terms of APD is limited, it is advisedly that this work is not reported at this time as size determination of arboviruses, but rather as their behavior on filtration.

The following technique has been generally used. A virus suspension is made consisting of a 10^{-2} suspension of infected newborn mouse brain tissue, using an electrical blendor; with group C viruses, infected whole blood or serum are used instead. The diluent is 0.02 M phosphate buffer in physiological saline, pH 7.2, to which is added 0.75 percent bovine plasma albumin. Fifty to 60 ml. of the viral suspension, freshly prepared, is centrifuged in a Spinco at 15000 r.p.m. for 30 minutes (Model L, Type 40 rotor). The supernatant is then filtered by pressure, in succession through filters of APD 450, 220, 100 and 50 mμ; at each level a sample about 5 ml. in volume is secured for titration. The least volume emerging through the last filter is 10 to 15 ml. The original suspension, the supernatant following centrifugation and each successive filtrate are titrated by the intra-

cerebral route in 2 to 4-day-old mice.

Since, with few exceptions, all the viruses tested were of a size that would not be materially excluded by a membrane with an APD of 450 mμ., the summary presented in the accompanying table has been prepared by referring the titer of the filtrates through 220, 100 and 50 mμ to that of the 450 mμ one; the latter has therefore been considered in this table as the "original working material". This has been done in order to have in all instances a uniform viral suspension to begin with, with no mixed-size tissue particles or aggregates that might be present in the original blendor material or even in the supernatant.

In addition to agents whose sizes are unknown, viruses are included - not exclusively arboviruses - the size of which has been reported in the literature, determined by various techniques; it is anticipated that they will act as known reference guide-posts.

This material is presented here without discussion or attempt to interpret it, for there are still other aspects of the work in progress, one of them being the influence of other diluents than the one used on the filterability. It is apparent, however, that not unexpectedly, there are considerable differences in the filterability of the arboviruses under the present conditions. Group B viruses and, it appears also some in group A, pass through a 50 mμ membrane with no significant loss in infective titer; other agents, as WEE, Bunyamwera, Wad Medani and perhaps Sindbis and EEE, go through a 100-mμ membrane with no loss but they suffer a significant loss after filtration through a 50 mμ pad.

Nyamanini virus has shown on two or three occasions when tested a striking behavior. Centrifugation followed by filtration through a 450-mμ membrane has reduced the titer from the original viral suspension, just emulsified brain tissue with no other handling, from $10^{5.5}$ or 10^{6} LD_{50}/0.02 ml. to $10^{1.8}$ or $10^{1.9}$ LD_{50}/0.02ml.; the residual virus being totally removed by filtration through a 220-mμ pad. Such behavior, if taken at face value, would indicate a very large particle. The matter is still under investigation. (J. Casals)

Filtration of arboviruses through Millipore Membranes

Virus (strain)	log LD_{50}/0.02ml in filtrate				Significant* log difference	APD of filter excluding $10^{1.7}$ or more LD_{50}
	450 mμ	220 mμ	100 mμ	50 mμ		
Dengue 2 (NGB)	4.8	4.9	5.0	4.6	0.2	F <50
Ntaya (prototype)	5.3	5.3	5.5	5.3	0	"
MVE (Prototype)	7.0	7.0	7.2	6.4	0.6	"
SLE (Parton)	7.0	6.9	7.0	6.6	0.4	"
SLE (Parton)	1.3	1.6	0.9	0.8	0.5	"
Chikungunya (Ross)	5.6	5.5	5.6	5.2	0.4	"
Sindbis (Eg Ar 339)	6.1	6.0	5.4	4.6	1.5	F ≤50
EEE (prototype)	7.4	7.4	6.7	5.9	1.5	F ≤50
WEE (McMillan)	7.0	7.5	6.6	4.6	2.4	50< F <100
WEE (McMillan)	3.9	3.9	3.0	1.0	2.9	"
Bunyamwera (prot.)	6.3	6.0	5.5	2.8	3.5	"
Wad Medani (Ar 492)	5.0	5.0	4.0	# 0	5.0	"
Wad Medani (I 673)	4.4	4.5	3.5	0	4.4	"
Wad Medani (J.#2)	4.1	3.5	2.6	0	4.1	"
Chenuda (Ar 1152)	5.0	4.5	3.5	0.6	4.4	50< F ≤100
Caraparu (An 3994)	3.1	1.9	0	0	3.1	100< F <220
Marituba (An 15)	3.6	3.1	1.9	0	1.7	"
Murutucu (An 974)	4.7	4.1	1.5	0.8	3.2	"
Oriboca (An 17)	2.5	2.0	0	0	2.5	"
Bwamba (prototype)	4.5	4.4	1.4	0	3.1	"
Colorado T.F. (Condon)	4.4	4.5	0.2	0	4.2	"
IG 619	3.6	3.6	0	0	3.6	"
IG 690	4.2	3.1	1.2	1.1	3.0	"
Kaisodi (IG 14132)	3.8	3.6	1.7	1.2	2.1	"
Thogoto (2 A)	3.1	2.6	0.6	0	2.5	"
Tribec (original)	5.0	4.9	1.0	0	4.0	"
Hughes (DT)	2.5	1.6	0	0	2.5	"
Nyamanini (AR 1304)	2.3	0.5	0	0	1.8	220< F <450
Reovirus 3 (Tr 23421)	5.4	5.3	4.0	0.6	4.8	50< F ≤100
NDV (98)	1.9	0	0	0	1.9	220< F <450

*Significant log difference is the arithmetic difference between the log of the virus in the 450 mμ filtrate and that of the first subsequent filtrate which differs from it by 1.7 or more. With viruses showing no such value, the difference entered in the column is that between the logs of the 450 mμ and 50 mμ filtrates.

#0, indicates that no virus was recovered from the material. While arithmetically incorrect in the context of the table, the figure 0 has been used to illustrate more clearly the origin of the differences in column 5.

Study of Egyptian Bird Sera

In a collaborative program with Drs. George Watson and John Hubbard of the Smithsonian Institution, virus isolation attempts were carried out from bloods of birds migrating from Europe to Egypt. Birds were bled immediately upon arrival in Egypt following trans-Mediterranean flight. More than 300 specimens from the fall, 1966 migration have been inoculated into baby mice. Three viruses have been established to date. The three agents cross-react by CF. The prototype, Eg B 90, is filterable and DCA sensitive.

Identification of Eg B 90 is still in the preliminary stages; however, a CF cross-reaction with SA An 4511 has been noted. No CF reaction was detected with 51 other arboviruses. SA An 4511 was isolated in South Africa from organs of a yellow-eye canary (*Serinus mocambica mocambica*) in 1959 by workers of the South African Medical Research Institute. Schmidt at USNAMRU III has isolated several viruses from Egyptian birds, and when these viruses become available at YARU in viable form, comparisons will be made with them. (R. E. Shope)

The Isolation of a Virus Belonging to the California Group of Arboviruses from *Aedes abserratus* (Felt and Young) Collected in Simsbury, Connecticut

Early in the spring of 1966 a study of the spring *Aedes* was initiated in the environs of the author's home. Larvae were first encountered on March 19th which were identified as *Aedes trichurus* and *A. stimulans*. The *A. trichurus* larvae were already in the second stage. First-stage larvae of *A. excrucians*, *A. canadensis*, *A. fitchii* and *A. abserratus* were collected between March 26th and March 30th. Due to the prolonged cold temperatures encountered in April larval growth was slow and the first natural pupae were not collected until April 21st. These proved to be *A. trichurus*. On April 25th pupae *A. stimulans* were found, and between May 4th and May 8th first pupae of *A. abserratus*, *A. excrucians*, *A. fitchii*, *A. canadensis* and *A. cinereus* were taken in approximately that order.

Light trapping was started on May 14th but no mosquitoes were taken. On May 17th one ♂ *A. abserratus* was collected and on May 21st four ♀ *A. abserratus* were taken. However, total mosquito captures were few during the rest of May, and these were mostly males. Commencing June 3rd, captures increased sharply, averaging a little better than 30 ♀ mosquitoes/trap night. These were identified and stored at -65° pending inoculation. Subsequently, pools were made by species,

covering three to four-day intervals, and were ground up for inoculation into suckling mice, intracerebrally. Because of the difficulty of separating adult females of A. excrucians, A. fitchii and A. stimulans they were lumped together in a group which we have called "exfitchulans" for convenience. As a biproduct of this light trapping, reasonably large numbers of Culicoides species were collected and pooled.

From only one of the 46 pools of mosquitoes and from none of the 27 pools of culicoides was virus isolated. The positive pool contained 20 A. abserratus of which 18 were caught between June 27th and June 30th. The other two specimens, the last collected of the season, were trapped July 7th. Eight one-day-old mice were inoculated i.c. on September 14th. These remained well for four days. On September 19th, two were sick in the morning and by noon two more. These were sacrificed for passage. The following day two of the remaining four were dead, the other two sick. After several passages the incubation period was somewhat reduced, the mice dying on the third and fourth days.

As soon as the virus was established, infected baby mouse brains were homogenized in nine volumes of saline and tested as an unknown CF antigen against sera of known viral agents. It became immediately apparent that the isolate belonged to the California group of viruses. This has been confirmed repeatedly both with the Simsbury antigen and sera prepared from it. As yet insufficient studies have been completed to determine its exact position in the group, but preliminary indications would suggest that it is not identical with any of the others with which it has been compared. (Loring Whitman)

The following table gives the total number of insects collected and inoculated into suckling mice.

	Specimens	Pools	# pools pos.
Aedes abserratus	184	6	1
Aedes "exfitchulans"	242	8	0
Aedes canadensis	52	7	0
Aedes cinereus	19	4	0
Aedes vexans	96	8	0
Aedes triserriatus	2	1	0
Anopheles punctipennis	1	1	0
Mansonia perturbans	26	2	0
Culiseta morsitans	1	1	0
Culex spp.	158	6	0
Culex territans	1	1	0
Uranotaenia sapphirina	2	1	0
	784	46	1
Culicoides spp.	2540	27	0

REPORT FROM THE DEPARTMENT OF ZOOLOGY, PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNSYLVANIA

A grant to train students in the study of birds as reservoirs of disease is in its fifth year of operation at Pennsylvania State University. Primary emphasis has been on wild bird populations of a 132-acre tract of mixed forest habitat near the campus. Mist nets are arranged in a square grid of 64 nets. Birds are banded and blood is taken from selected species. Serological tests for activity of viruses are nearly completed, and blood smears have been examined for protozoan parasites. Mosquito populations of the tract are also under study.

Bird-banding continued through mid-October. Two thousand three hundred and seventy-four birds representing 86 species were captured. From these, 932 blood smears and 795 serum samples were obtained.

Routine examination of blood smears continues. Forty-four house sparrows (*Passer domesticus*) were captured in the vicinity of the campus horse barns and bled. No malarial parasites were found in the blood smears.

In a manuscript, a model was introduced which enabled calculation of the amount of time that a slide with a known percentage of cells infected with malarial parasites must be observed in order to be confident that a parasite will be found. If the amount of time required to diagnose one bird by the subinoculation technique were used in screening a slide from the wild bird, the investigator would find 95% of the infections at a parasite level of 1 infected cell per 150,000 erythrocytes. Evidence was presented to show that the subinoculation method is less than 95% efficient at this low level of infection, and it was concluded that use of subinoculation in survey work is inefficient and impractical.

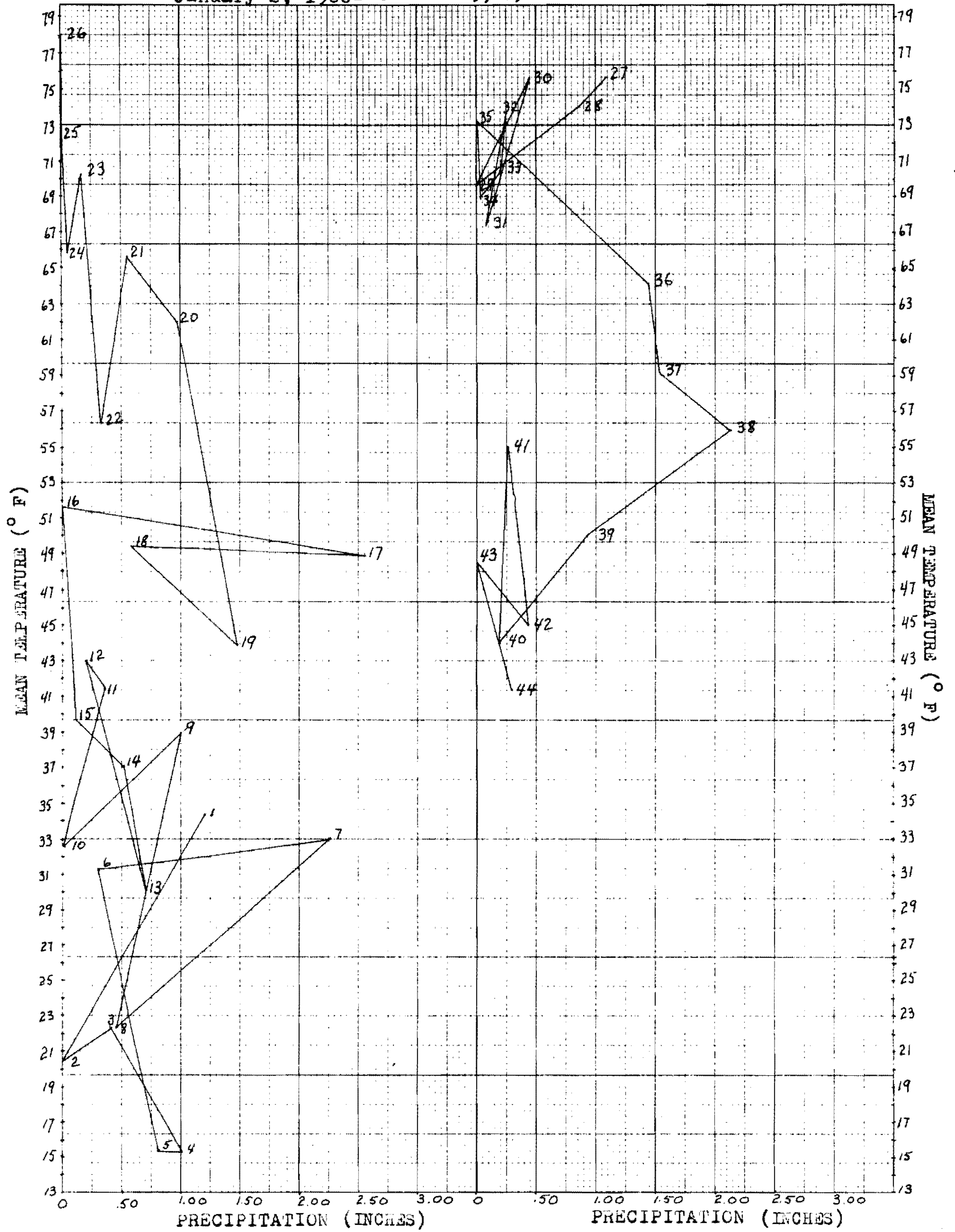
Weekly samples from the one permanent pond in the woodlots produced only Anophelene larvae and pupae from July 2 to July 29. From July 29 to September 2, larvae and pupae from both Anophelene and Culex mosquitoes were found, with a peak abundance during late August comprised primarily of *C. restuans*.

Plastic cups. to simulate treeholes, were fastened to trees and supplied with fresh water. These were readily colonized by *Aedes triseriatus* after start of the work on July 7, and this continued through September 2.

Temperature and precipitation data for the State College area were plotted by weekly intervals to form an annual hythergraph for each of the years, 1962 through 1966. Figure 1 shows the form of these data for the first 44 weeks of 1966. It is possible by observing the grouping of points on these graphs to divide the year into seven seasonal periods. These vary in length from year to year and should provide suitable intervals for grouping of data on birds, mosquitoes and viruses more strictly in accordance with the true seasons. From the 1966 records the periods were as follows: Hibernal, weeks 1-8, January 2-February 26 (56 days); Prevernal, weeks 9-15, February 27-April 16 (49 days); Vernal, weeks 16-19, April 17-May 14 (28 days); Estival, weeks 20-26, May 15-July 2 (49 days); Postestival, weeks 27-35, July 3-September 3 (62 days); Serotinal, weeks 36-38, September 4-24 (21 days); Autumnal, weeks 39 at least through 44, September 25 to November 5 or longer (42 days or more). The year's end Hibernal followed the Autumnal. (David E. Davis and P. Quentin Tomich)

Figure 1 - Hythergraph by weekly intervals for State College, Pennsylvania, for 1966.

Hythergraph by weeks- State College, Pa.
January 2, 1966- October 29, 1966



REPORT FROM FLORIDA STATE BOARD OF HEALTH, JACKSONVILLE, FLORIDA

The state-wide arbovirus surveillance program has been in operation for the past four years since the SLE epidemic in the Tampa Bay area. The laboratory examination of human, biological and entomological specimens, collected throughout Florida, serves to maintain a current index of arbovirus activity.

EE was diagnosed in a four-week-old girl born in Jacksonville, Florida in August, 1966. Beginning at the age of seven days, she made three brief trips from Jacksonville to Darien, Georgia, where on each occasion she sustained numerous mosquito bites. She was hospitalized at the age of one month because of signs and symptoms of central nervous system disease including seizures. Serologic confirmation of acute EE infection was obtained by HI, CF, and SN tests. No antibody was demonstrated to WE or SLE. Serum obtained simultaneously from the mother showed no antibody to EE, SLE or WE. This was the only known human EE case diagnosed on the eastern coast of the United States in 1966.

In September, there was an outbreak of EE in a flock of 65 pheasants in northern Florida. Thirty percent of the seven to ten-week-old pheasants died within three days. HI antibody titers of 1:80 ->1:640 were demonstrated in all but one of 20 surviving birds bled three weeks after the outbreak.

Extensive EE activity was evident in the central portion of Florida from May to September. During this short period, 124 horse cases were diagnosed as EE by clinical and/or serologic studies. A total of 145 horse cases were reported for the year. EE virus was isolated from a horse brain and also from an immature Loggerhead shrike.

To date, 32 arboviruses have been isolated from 1497 of the 2200 pools of mosquitoes collected in 1966. These include: seven EE, two WE, four Bunyamwera Group, 13 California Group, and six Hart Park-like.

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

The dispersal of Culex nigripalpus, the mosquito vector of SLE virus in the 1962 Florida epidemic, was studied in 1965 by releasing over 130,000 females marked with a radioisotope. The results were considered questionable when it was found that the marking of the released specimens had apparently been high enough to reduce their longevity. The isotope used, P-32, had been given to the last instar larvae as inorganic phosphate. Recent studies have confirmed that a large part of the radioactivity in the adult female is lost at oviposition (31 to 44 percent in the first egg raft and 20-33 percent in the second raft, the percentage actually increasing as the larval dosage is reduced). Enough radioactivity to last the lifetime of a female but not enough to affect longevity seemed to call for low marking within a very narrow range. This problem has now been largely sidestepped by feeding the larvae with radioactive yeast. Adults so marked have much higher activity for the amount of isotope used, and there is accumulating evidence that they are less damaged. In fact, it seems probable that most of the biological indications of radiation damage so far uncovered have been due to the unnecessarily high levels of radioactivity required to mark adults by feeding the larvae with inorganic phosphate. Work is being continued on the use of marked yeast.

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

Isolation of St. Louis Virus from Mosquitoes:

A strain of St. Louis virus was isolated from 539 Culex nigripalpus collected at the Caymanas Estate during the month of July 1966 by means of a CDC light trap. All of the mosquitoes were processed in one pool and inoculated in suckling mice from which an agent was isolated. The isolate was chloroform sensitive. It produced haemagglutinin at pH ranges 6.0 - 6.6 and was subsequently identified as a strain of St. Louis encephalitis virus in a neutralization test.

Thus far St. Louis virus has been isolated on three occasions from

Culex nigripalpus collected in the Caymanas area. On one occasion, the virus was isolated from the serum of a nestling nightingale (Mimus polyglottus) caught in the same area.

Bird Sera:

During the last quarter several paired bird sera were tested for EEE antibody. These birds were bled between June 1964 and December 1966. (See Inform ation Exchange Report for September 1965). Sera from one bird, Vireo altiloquus (John-to-whit) (AH-59) caught and bled at Amity Hall, St. Thomas on June 15, 1964 and again on June 8, 1966 neutralized 40LD_{50} of EEE virus. Both sera were diluted at 1:20.

Cache Valley Virus:

Studies with this virus currently being undertaken at our Laboratory are:

(1) To determine the laboratory host-range of this virus. This includes animals and tissue culture investigations.

(2) Testing the virus for production of interferon in tissue culture.

(3) Column Chromatography involving Cache Valley virus

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

Bush Bush:

No virus isolations were made from any source material from this area since the two Catu strains encountered in January. Rodents remained very scarce and during the second half of the year the most important vector Culex portesi was also less abundant than in previous years.

Turure Forest:

This forest area near the town of Sangre Grande was studied inten-

sively during 1966. So far VEE, Caraparu, Guama, Bimiti, Catu, EEE, Wyeomyia and Aruac virus strains have been recovered from the area. For the first five viruses sentinel mice and Culex portesi were the most frequent sources. EEE was isolated only from sentinel mice, Wyeomyia only from Limatus flavisetosus. The one Aruac strain came from Psorophora ferox. A survey for the distribution of Culex portesi is in progress. Long-range plans for study in the area are being developed. A field laboratory will be constructed in the area in the first months of 1967.

Cedros Area:

Monthly expeditions of one week's duration, each centering on the Lillette swamp on Trinidad's south coast, have so far not yielded any virus containing material.

EEE Surveillance:

This program involving bleeding, banding and recapture of birds has been stopped after three years of continuous operation. Over 3000 birds were tested for EEE neutralizing antibodies, 2295 were tested for circulating virus also. It was found that this program did not give sensitive indication of EEE activity and consequently, it has been abandoned for the time being. EEE virus was isolated in 1966 from Culex taeniopus from Vega de Oropouche and from sentinel mice exposed in Turure Forest. An isolation was also made from the serum of the Bananaquit, Coereba flaveola, caught in Brazil Village, but re-isolation failed, raising some doubt on the validity of the isolation.

EEE virus was once again active on the coastal strip of Guyana and was isolated from the serum of two horses. From the serum of a third horse, Cache Valley virus was isolated. More than 100 horses died during the outbreak. (L. Spence, E. Tikasingh, J. Davies and A. H. Jonkers)

REPORT FROM THE VIROLOGY DEPARTMENT, INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS, CARACAS, VENEZUELA

In October 1966 occurred a VEE epidemic in the city Guasipati and in the village Callao, Estado Bolívar, south of the Orinoco, in southeastern Venezuela. About 200 cases, of which about 150 were children, with

one fatality (child) were reported. The epidemic was apparently preceded by an epizootic of donkeys in the same area some months earlier. O. Suárez went into the area (middle of October) and collected 2532 mosquitoes with light traps in the surroundings of the city Guasipati and the village Callao. There were predominantly Aedes aegypti and Culex quinquefasciatus. These 2532 mosquitoes were divided into 143 lots. None of these contained any virus, with the possible exception of one fully engorged Phlebotomus specimen. This is under further investigation.

Eight litters of baby mice were exposed in eight houses with patients in Guasipati, before capturing mosquitoes. One baby mouse of a litter exposed in a house with two very sick patients (40°C.) became paralyzed within 24 hours. The following three mouse brain passages killed readily within 24-30 hours and neutralization test revealed VEE.

Two hundred and fifty-six mosquitoes were collected in stone houses in Guasipati with patients with high fever. Of the 256 mosquitoes collected in the houses, there were 181 Aedes aegypti, 72 C. quinquefasciatus and one Ae. serratus. Three of the four collected serum samples of patients contained VEE. Of the mosquitoes collected in the houses, three about equal lots consisting of Ae. aegypti and C. quinquefasciatus were permitted to feed in the campamento in Guasipati on one litter (eight baby mice) each. One of the lots (35 Aedes and four Culex) caused paralysis of all babies within 24 hours. The following mouse brain passages killed readily, and neutralization tests revealed VEE. The same lot was brought to the lab at IVIC and permitted to feed on litters six, 11, 18 and 21 days later. The Aedes (11) transmitted the virus only at a six-day interval, as well as the two Culex. No virus was found in Aedes nor Culex dying during the experiment or killed after 21 days.

During this investigation a technician contracted VEE due to an accident in handling an ampule. O. Suárez also got VEE when checking a VEE neutralization test in the quarantine mouse room. Three hours after onset of fever, seven laboratory-reared unfed Ae. aegypti were permitted to ingest on the patient's arm, when the fever had reached 40°C. and 5.10^7 mouse LD_{50} were present per ml. of the patient's serum. These seven mosquitoes transmitted VEE six, nine and 12 days afterward readily to baby mice but not after 15 and 30 days, although virus was found 37 days later in the triturated mosquitoes. A second lot of 54 Ae. aegypti were permitted to feed on the patient 27 hours after onset of the fever, but no virus transmission occurred

in 20 days, although virus was found in the triturated mosquitoes after 32 days. Two lots of laboratory-reared C. quinquefasciatus were permitted to ingest on the patient nine and 30 hours after onset of fever, but no transmission took place. The first lot of Culex possibly contained virus after 30 days. This is under further investigation.

Lab experiments revealed that VEE is readily transmitted by Ae. aegypti but not by Culex from three to at least 40 days after feeding on viremic mice. The above investigation demonstrated clearly that Ae. aegypti can be of great importance in causing VEE outbreaks in villages and cities.

During November 1966 there was a small outbreak of Yellow Fever in two caserías about 25 kms. south of the sea port Puerto La Cruz, Estado Anzoategui. Three fatalities were reported. Immediate vaccination of about 40,000 persons in the area was carried out by the División de Fiebre Amarilla y Peste. O. Suárez visited the area at the end of the epidemic but could not find any mosquitoes due to a very extensive and effective fumigation program.

The following viruses kindly supplied by Dr. Leslie Spence and Dr. Karl Johnson were purified and demonstrated with the electron microscope: Manzanilla, Oriboca, Restan (J. Bastardo); Changuinola, Mucambo, Oropouche (L. González); Catu, Guamá and Bimiti (Th. Graf). All these viruses are spherical, having diameters between 44-59 mμ. and belong to class 1 of typical arboviruses. Junin, kindly supplied by Dr. K. Johnson, is also spherical, having a diameter of 60 mμ. and a much less pronounced surface than the much larger Tacaribe (85 mμ.).
(G. H. Bergold, Ph.D.)

REPORT FROM GORGAS MEMORIAL LABORATORY
PANAMA, R.P.

Transmission of Arboviruses to Hamsters By the Bite of Naturally Infected Culex (Melanoconion) Mosquitoes :

It has been previously demonstrated that the golden hamster is highly susceptible to a number of arboviruses and that such viral agents as VEE, Ossa and Madrid produce rapidly fatal infections in this rodent. Since some of these viruses are frequently isolated from Culex

(*Melanoconion*) mosquitoes captured in the Almirante study area, experiments were undertaken to determine whether wild-caught specimens belonging to this subgeneric group of mosquitoes were capable of transmitting these naturally acquired infections to clean golden hamsters.

Mosquitoes were captured at night with human bait in areas where VEE, Ossa, Madrid and Guamá viruses were known to be active. Captured culicines were maintained alive in plaster of Paris-lined jars and shipped by air to Panama City three times a week. Once in the insectary, *Melanoconion* mosquitoes were sorted according to species into bobbinet cages of 1 cubic foot capacity in numbers not exceeding 75. A golden hamster was introduced five nights a week into each cage and removed the following morning. Engorged mosquitoes in the bobbinet cages were also removed and placed in oviposition cages for colonization attempts. Twice during the day all cages containing mosquitoes were examined for dead or moribund insects which were removed, checked for correct identification and frozen for virus isolation attempts. If any of the exposed hamsters sickened or died a heart blood sample was obtained whenever possible and pieces of heart, liver and brain removed for virus isolation attempts. All hamsters that survived at least one month were bled out and the serum used to demonstrate appearance of specific antibodies against certain arbovirus which would indicate transmission of these agents through the bite of the species of mosquitoes involved.

A total of 11,598 wild-caught females of *Culex vomerifer* and 229 specimens of other species of *Culex* (*Melanoconion*) mosquitoes were allowed to feed on 54 laboratory-bred hamsters. Of the *C. vomerifer* given access to hamsters, 3,987 were observed to engorge on blood. A total of 18 transmissions of arboviruses were demonstrated in hamsters fed on by *C. vomerifer* mosquitoes and 1 strain of Ossa virus was obtained from a hamster fed on by *C. taeniopus*. Nine of the transmissions by *C. vomerifer*, 5 of Ossa virus and 4 of Madrid virus were identified by isolation of the agents from the tissues of hamsters fed on by these mosquitoes. The other 9 transmissions, five of Guamá and one each of Ossa, Madrid, Patois and Mayaro viruses, were demonstrated and identified by subjecting the serum of surviving hamsters to HI and CF tests against antigens of 27 different arboviruses. All five viruses transmitted were previously known to occur in the Almirante area, and four of them are commonly encountered there. The rate of transmission of all arboviruses obtained from *C. vomerifer* was of one arbovirus transmitted for every 222 females which engorged on the hamsters, which is a much higher infection rate than has been

obtained in the Almirante area through inoculation of wild-caught mosquitoes into suckling mice.

No viruses were obtained from dead mosquitoes removed from the cages, probably due to rapid decomposition of the insects because of the high relative humidity maintained in the experimental room. However, live *C. vomerifer* females from the same collecting lots as those used in the transmission experiments, yielded isolates of Guamá, Ossa, Madrid and VEE viruses when inoculated into mice in connection with another project, thus demonstrating the presence of these viruses in the *C. vomerifer* population used in the transmission experiments.

The high infection rates demonstrated in wild populations of *Culex (Melanoconion)* mosquitoes by the transmission method described above, points to this method as a feasible routine procedure to be used instead of direct inoculation of mosquitoes into mice. This is of particular significance considering the fact that at least some of the isolations obtained from inoculation of mosquitoes may be dead-end infections. Unless carefully weighed, results obtained from such isolations could lead to erroneous conclusions regarding the vector potential of the species of mosquitoes found infected. In the transmission method all the isolations obtained are the result of direct transmission by bite, thus offering indisputable evidence of the ability of a species of mosquito to pick up arboviral infections in nature and to transmit them by its bite to a susceptible vertebrate host. (Pedro Galindo and Sunthorn Srihongse).

Detection of Arboviruses by Sentinel Hamsters During Unfavorable Period of Transmission:

Previous work done in Almirante, a tropical rainforest area located in northwestern Panama, indicated that there were definite seasonal fluctuations in arbovirus activity. The months from February to April proved to be the period of lowest transmission of arboviruses. Of 33 virus strains recovered from sentinel mice in 1965-66, none was isolated during the months of February and March.

Young adult hamsters were successfully used as sentinels to detect VEE virus in Mexico by Scherer *et al* in July and August 1963. Laboratory experiments have shown that this animal is susceptible to VEE and many group C viruses which are known to be present in the Almirante study area. Therefore, a field experiment was set up to determine whether or not adult hamsters can be used as sentinels to detect dif-

ferent types of arboviruses during the season of lowest activity. Dr. Scherer very kindly made suggestions and joined us in person to initiate this program in Almirante in February 1966.

Encouraging results were obtained as early as a few days after the first group of hamsters were exposed. From February 15 to March 15, 1966 a total of 21 golden hamsters (Mesocricetus auratus) was exposed in 5 localities in the Almirante area at ground level or in the canopy of the forest. Of these exposed animals, 20 were either found dead or ill, mostly within 4 to 10 days. Viruses were isolated from 16 hamsters. Preliminary identification was performed in HI tests using infected suckling mouse serum as the hemagglutinating antigen. In this study, two isolates, both from hamsters exposed at ground level, were identified as VEE virus. Ten strains of Ossa and two of Madrid viruses were obtained, both at ground level and in the canopy. Another isolate, from a ground-level hamster, was identified as Patois virus. Beside these two groups of viruses, A and C, one isolate of Guamá group was recovered from the brain of a hamster exposed in the forest canopy at the edge of a swamp.

The identity of these isolates was confirmed by cross HI and CF tests. The demonstration of VEE virus activity was of interest since no isolations have ever been obtained in the Almirante area between February and May. The preponderance of Ossa virus at this time has been confirmed by the increased number of human isolations of this group C agent since January, accompanied by a reduction in the usual intensity of transmission of VEE virus.

Since the sentinel hamsters were left in the field until they were sick or died, feed-back of virus from viremic animals was possible. This was avoided in subsequent tests by moving sentinels to other areas. Furthermore, multiple infections in an individual hamster were also possible. Therefore, newly-isolated strains from these hamsters were tested as follows:

1. Both liver and brain antigens of each of the isolates were tested by CF technique with immune sera of 18 arboviruses previously encountered in this area.

2. Immune sera prepared from each of the isolates were checked by HI tests with 25 arboviral antigens and by CF with 6 other antigens.

Results of these two methods showed that mixed infection occurred in one instance; the Guamá isolate being accompanied by Ossa virus.

During the period of these studies, no viruses were isolated from a total of 48 litters of suckling mice exposed in the same localities once a week for 12-hour periods from January to March 1966.

In summary, 16 virus isolates were obtained from 21 hamsters exposed in a tropical rainforest area of Panama. These animals proved to be successful sentinels to detect VEE, Ossa, Madrid, Patois and Guamá viruses during the unfavorable period of transmission between February and March of 1966. (Sunthorn Srihongse and Pedro Gallindo).

REPORT FROM THE MIDDLE AMERICA RESEARCH UNIT (NIAID), PANAMA

Bolivian Hemorrhagic Fever:

Virus Isolation: A brief description of the work of a 7-man survey team was reported in the #14 Exchange. Small mammals were collected from areas in Bolivia, Brazil, Paraguay and Peru during the months of April to October 1965. Spleen and kidneys were preserved in liquid nitrogen from about 1450 animals for subsequent virus isolation attempts in suckling hamsters. Since it was not feasible to test all specimens, selections were based on the following considerations:

1. The genus *Calomys* is known to harbor virus in Bolivia and Argentina.

2. San Joaquín, Bolivia is a known epidemic area.

3. Isolations of Tacaribe-Group viruses have been reported from the genera *Akodon*, (closely related to Zygodontomys) *Oryzomys*, and *Neacomys*, all of which, like the laboratory hamster, are members of the family Cricetidae.

4. Sera from wild-caught *Proechimys* from epidemic areas occasionally contain antibodies but, to date, virus has not been isolated from this animal, either wild-caught or laboratory inoculated.

Thus virus isolation attempts were made from all specimens from (a) the genera Calomys (208), Oryzomys (59), and Zygodontomys (7); (b) all animals collected in San Joaquin and its immediate environs, Calomys (25), Proechimys (50), Oryzomys (8), Zygodontomys (2), Marmosa (6), Holochilus (1); (c) a geographically selected number of cricetine rodents (106) not yet identified to genus.

No agent was isolated from Proechimys, Zygodontomys, or from the rodents so far classified only as Cricetidae. With the exception of a single hamster pathogenic agent isolated from an Oryzomys captured in the Madre de Dios area of Perú, all agents were isolated from the genus Calomys. These results are summarized in Table I. The three isolations from Calomys near San Joaquin have been found by neutralization test to be Machupo virus. A total of fifteen hamster pathogenic agents have been isolated from Calomys trapped in two areas in Bolivia and one in Brazil. Complement fixation tests indicate that these viruses are members of the Tacaribe Group but preliminary neutralization results show no relationship to Machupo, Junín, Tacaribe or Amaparí.

Human Infection: Serologic evidence of human infection with Machupo virus, in association with Calomys, was found in a new area in the Beni Province following reports of a febrile illness occurring in June 1966. The area, Rio Negro, is comprised of scattered estancias, situated approximately 100 km. to the southeast of San Joaquin. Neutralizing antibodies to Machupo virus were found in 27% of 71 human sera and in 30% of 47 Calomys sera. CF titers of 16 or greater in human sera suggest that half of the positives were associated with infections acquired in the past year. Five of the sera with neutralizing antibody contained no CF antibody suggesting old infection. Thus it appears that HF may have been active in Rio Negro prior to 1966 but that a recent burst of infection has occurred.

Virus studies: Experiments designed to investigate some biophysical and biochemical properties of Tacaribe-group viruses have been initiated. Junín virus (XJ) grown in VERO cells and containing 1.4×10^7 PFU/ml was centrifuged in a SW-39 Spinco rotor at 35,000 rpm for 24 hours on a 10-60% sucrose gradient column. Ten fractions of equal volume ($\pm$ 0.5) were collected by puncture of the tube and densities were measured by refractive index. Fractions were then assayed for plaque formation on VERO cells and for complement fixing activity.

Biological activities recovered were calculated as percentage of total activity recovered. Results of one experiment are shown in Figure 1. Peak infectivity (3.2×10^6 PFU/ml) was found at a density of 1.1642

g/ml. whereas maximum CF activity (1:32) occurred at 1.0845 g/ml. There was a strong degree of dissociation of the two properties.

These data suggest that suspensions of Junin virus contain at least two different antigenic components, one of which cannot be directly related to the infectious virus particle. Further studies are being made to define more clearly these moieties and to relate them to group or strain specific antigens.

Central American Human Survey for Arbovirus Antibodies

Although their natural history is not well understood, vesicular stomatitis viruses (VSV) have been returned to the list of arboviruses by several workers, because of fragmentary information incriminating mosquitoes, phlebotomus and possibly mites as potential vectors. Neutralizing antibodies to New Jersey and Indiana serotypes have been found in human sera in Panama. Before attempting more detailed ecological studies it was decided to try to better define the role of these agents in human infection. The incidence of such antibodies as well as those for Cache Valley virus are being determined by testing sera from the 6 Central American countries collected as part of a (NIH-OIR) - (INCAP) nutrition survey currently in progress.

To date a total of 2310 sera of persons more than 14 years old from the first 3 serum collections (Guatemala, El Salvador, Nicaragua) have been screened in a plaque reduction test using one serum dilution, a single virus dose and VERO cell cultures grown in disposable plastic trays. (A description of this micro-method for arboviruses is to be published shortly by Earley, Peralta, and Johnson). Using 95% plaque reduction as indication of presence of neutralizing antibody, we have found that 57% of all sera tested so far have antibody to New Jersey type VSV, and 20% to Indiana type. Few sera show antibody to Cocal virus and these apparently as a result of cross-reactivity.

A summary of results by country is shown in Table 2. In individual communities up to 100% of sera tested were VSV-N.J. positive and 70% Indiana-positive, although in some localities the viruses were apparently absent. Taking all sera from one country together, the percentage of positive reactors was found to be between 49 and 63% for the N.J. type. In almost every community the incidence of N.J. antibody exceeded that of Indiana antibody. Prevalence of Cache Valley virus antibody was found to fall between that found for the 2 types of VSV in all three countries. Detailed tabulations are not yet complete. Sera from persons of all ages in selected communities will be studied

in the next phase of this investigation in order to learn more about the epidemiology of these viruses.

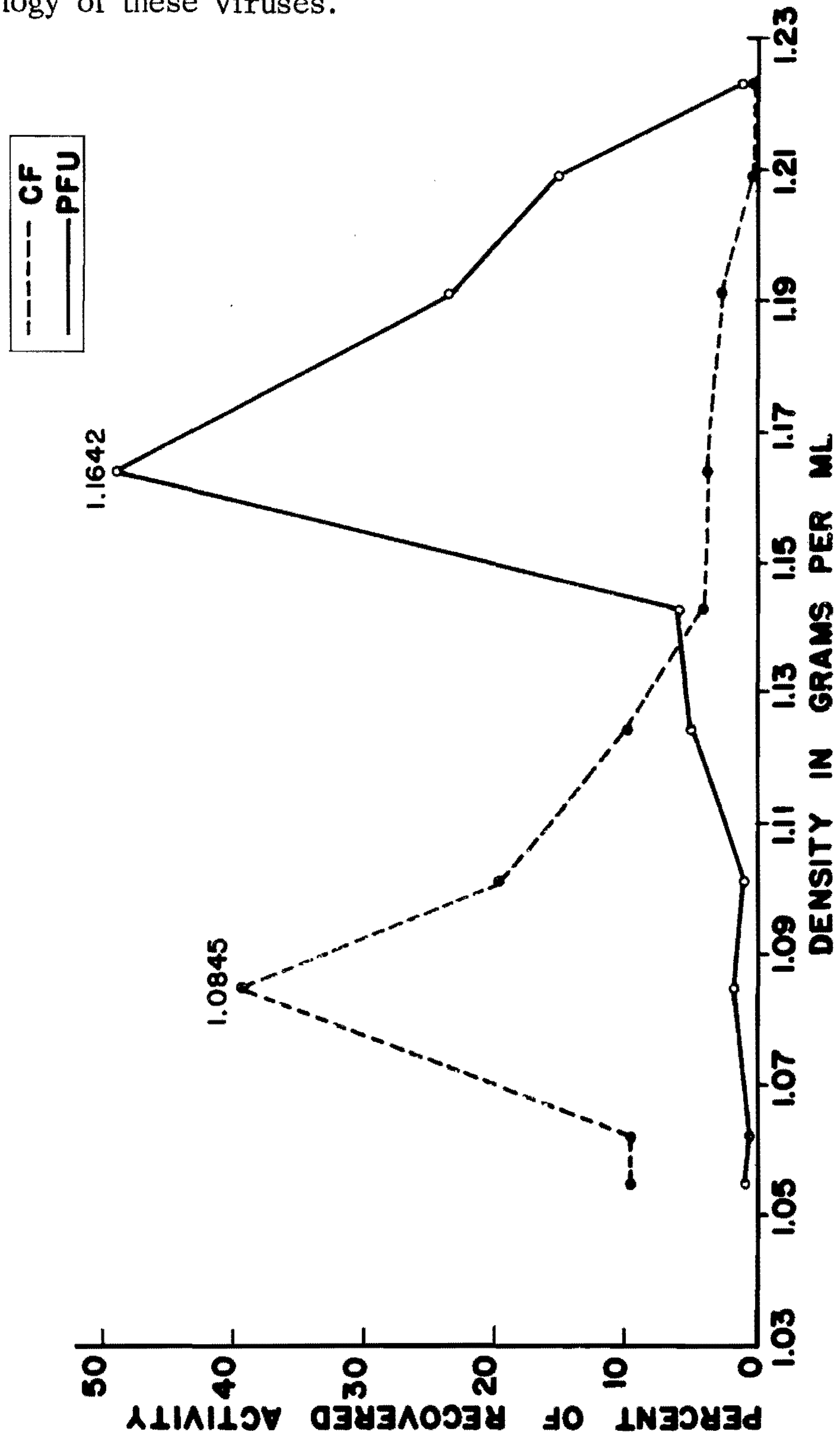


Table I

Virus Isolation from <u>Calomys</u> kidney

Country	State	Locality	Number Tested	Virus not Isolated	Virus Isolated	
					Machupo	Tacaribe Group Untyped
Bolivia	Beni	San Joaquin & Environs	28	25	3	
		Ignacio de Moxos	2	2		
	Santa Cruz	Warnes	8	5		3
		Ignacio de Velasco	115	104		11
Paraguay	Boqueron	Guachalla	9	9		
Brazil	Mato Grosso	Caceres	13	13		
		Corumba	33	32		1
		Total	208	190	3	15

Table 2

Incidence of Neutralizing Antibodies to New Jersey and Indiana Types VSV in Three Central American Countries

Country	No. Communities tested	Minimum & Maximum Incidence in Single Community		No. sera tested	Incidence of Antibodies in Entire Group	
		VSV-N.J.	VSV-Ind.		VSV-N.J.	VSV-Ind.
Guatemala	39	0 - 96%	0 - 70%	907	49%	23%
El Salvador	29	17 - 88%	0 - 44%	678	63%	21%
Nicaragua	30	10 - 100%	0 - 48%	725	62%	16%
All 3	98			2310	57%	20%

REPORT FROM THE INSTITUTO DE VIROLOGIA DE CORDOBA, ARGENTINA

Junín Virus Studies:

Human Cases. In 1966, we recorded 65 clinically suspected cases of Argentinian Hemorrhagic Fever in the Province of Córdoba. Forty-three cases were confirmed by Junín virus isolation and/or serological conversion (CF), 3 had a doubtful serological conversion, 12 were negative and 7 were not studied. The monthly distribution of confirmed cases was as follows: 2 in February, 3 in March, 6 in April, 13 in May, 13 in June, 5 in July and 1 in September.

Junín virus was isolated from 16 human cases. Thirty-four blood specimens, 28 urine samples and 36 pharyngeal swabs from confirmed cases were studied, from which 13, 2 and 5 virus strains were recovered respectively. Three isolations obtained simultaneously from blood and pharyngeal swab came from patients who presented gingival bleeding, hematemesis and/or hemoptysis which could explain the presence of Junín virus in the throat swabs. The same can be said of the isolations from urine specimens.

It seems of importance the presence of Junín virus in urine and pharyngeal secretions as they can be regarded as possible sources of infections, although up to the present interhuman transmission has only occasionally been demonstrated.

Rodent Studies. A simple and time-saving procedure for direct identification of Junín virus in natural infected wild rodents was followed. It consists of obtaining crude antigens with the brain, spleen, liver and kidney of each rodent, and to test their ability to fix complement against a hyperimmune serum of Junín virus. The results are Tacaribe Group specific. Thus, 48 rodents (36 *Calomys laucha*, 10 *Akodon azarae*, 1 *Oryzomys flavescens* and 1 *Mus musculus*) live trapped in the endemic area of Córdoba Province, were investigated. Junín virus (CF antigen) was found in the brain and viscera from two *Calomys*. Simultaneously virus isolation and reisolation attempts in baby Swiss mice yielded Junín virus from four *Calomys*, two of which were the same positive rodents in the direct identification by complement fixation test. (Marta S. Sabattini, Lola E. González and Juan C. Fain Binda).

REPORT FROM THE ARBOVIRUS EPIDEMIOLOGY UNIT, MICROBIOLOGICAL RESEARCH ESTABLISHMENT, PORTON, SALISBURY, WILTS, ENGLAND

Studies on mosquito-transmitted encephalitis in Sarawak have continued. A further 3-1/2 month expedition was made from September to mid-December, 1966. The object was to test the hypothesis that Japanese encephalitis virus is maintained throughout the year by pigs and Culex gelidus but that the greatest risk of infection to man occurs when the ricefields are flooded and provide large populations of Culex tritaeniorhynchus and related species. For this reason work has been concentrated around piggeries and ricefields. Approximately 88,000 mosquitoes were collected, identified and pooled by species for virus isolation attempts. About 23,000 were C. gelidus and about 35,000 were rice-breeding Culex species. So far only 40,000 mosquitoes in pools of 100 have been examined for virus. Twelve strains of virus have been isolated in suckling mice and 6 in chick embryo tissue culture giving an isolation rate of 1 virus for every 3000 mosquitoes. Some of the strains have not yet been identified. Six strains were isolated from 126 pools of C. gelidus, (1 in 2,100), four from 68 pools of C. tritaeniorhynchus (1 in 1,700) and two from 34 pools of C. pseudovishnui (1 in 1,700). A further probable virus isolate was obtained from Mansonia annulifera, probably the commonest man-biting mosquito in Sarawak. 605 human sera and 128 animal sera were also collected, but antibody studies have not yet been carried out.

The MS 50 strain of Bunyamwera virus isolated from Aedes curtipes on the previous Sarawak expedition has been studied in more detail. Earlier serology had shown it to be closely related to Batai virus. This has been confirmed by gel precipitation but the two viruses are antigenically distinguishable. Paired sera from cases of mild febrile illness were examined for the presence of antibody. Two patients were found to have a rise in MS 50 neutralizing antibody. Human antibody surveys on sera collected in various habitats in Sarawak show a low incidence of MS 50 antibodies suggestive of annual infection rates of 0.09% to 0.9% in various areas. Highest rates were found in the coastal fringe and in inland forest. A. curtipes is common in the coastal swamp but absent in inland forest.

Louping ill studies have continued in Ayrshire. No virus was isolated from ticks and small mammals collected during 1965, but the infection rate in sheep remains about 30% per annum. Small mammals and ticks collected in the infected areas in 1966 have not been processed

yet.

In collaboration with Dr. H. E. Webb and Prof. G. Wetherley-Mein at St. Thomas's Hospital, London, experiments have continued on infection of cancer patients with Langat virus. The candidate vaccine strain derived by Dr. Winston Price (Johns Hopkins) was given to six patients at least five of whom showed very good antibody responses. There were no symptoms or signs attributable to the infection although two had viraemias. The results are summarized in the Table.

Provided its safety can be established, Langat virus is a very good candidate as a live vaccine against the whole of the tick-borne complex of group B arboviruses.

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VIRAEMIA AND ANTIBODY RESPONSES IN PATIENTS INFECTED WITH THE WINSTON PRICE STRAIN OF LANGAT VIRUS

DAYS AFTER INFECTION	PATIENTS																	
	RF			TM			ML			DD			ATW			WR		
	VIRAEMIA	HI	NEUT	VIRAEMIA	HI	NEUT	VIRAEMIA	HI	NEUT	VIRAEMIA	HI	NEUT	VIRAEMIA	HI	NEUT	VIRAEMIA	HI	NEUT
PRE		<1/10	0		<1/10	0		<1/10	0		<1/10	0		<1/10	0		<1/10	0
3				+			–											
4	–									+			–			–		
6				+			–			+			–			–		
7	–																	
8				+			–											
9	–									–			–			–		
10				–			–									–		
11	–									–			–					
13				–			–											
14	–												–			–		
16													–					
20		1/10			<1/10	NEG		<1/10	NEG									
21											1/40	1/80					<1/10	NEG
23		1/10	NEG															
27					1/80	1/80		1/10	NEG									
28											1/40	1/80		<1/10	NEG			
31																	1/40	1/320
32		1/80	1/320															

REPORT FROM THE LABORATOIRE DES ARBOVIRUS, INSTITUT PASTEUR, PARIS

Ecology of Arbovirus in Southern France:

a). Serological surveys. The serological surveys on human subjects have been extended to the areas east and west of Camargue where West Nile virus was isolated for the first time in 1964. They showed a high incidence of positive subjects for group B antibodies in the "Alpes-Maritimes", near the Italian border and in Corsica.

Serological examination of horses confirmed the preliminary results already reported here; in Camargue a large proportion of horses have West Nile antibodies, all of them are NT positives for Tahyna. A large number of horses from other regions of France was studied as a control group and was found totally negative.

HI reactions were also performed on bird and rodent sera and gave a small proportion of positives for group B. The collection of blood on paper discs and treatment of the discs gave very good results and a detailed description of the technique used is available on request.

b). Virus isolations. West Nile virus was again isolated in 1965 from several pools of the mosquito Culex molestus and also from the central nervous system of a foal after encephalomyelitis (Panthier, Hannoun, Oudar, Beytout, Corniou, Joubert, Guillon et Mouchet, C. R. Acad. Sc., 1966, 242, 1308).

Tahyna virus was isolated in 1965 (Hannoun, Panthier and Corniou, Acta virol., 1966, 10, 362) and 1966 from 5 pools of Aedes caspius collected in different parts of Camargue and also from the blood of three indicator rabbits (out of 6) exposed in September 1966. The same experiment had been made in June 1966 but although 2 rabbits out of 12 converted to positive serology, the virus isolations attempts had failed. Using the serum of one of the virus-yielding rabbits, a strain of Tahyna virus was isolated by intraperitoneal inoculation of newborn mice and is carried by this "extra-neural" route of passages on these animals similarly to the "extra-neural" strain obtained by Bardos on hamsters.

Experimental Inoculation of West Nile Virus to Horse:

In collaboration with the "Ecole Vétérinaire de Lyon" (Pr. Joubert and

Oudar) experimental inoculation of West Nile virus (horse strain) was made to horses in order to try to reproduce the disease observed under natural conditions. This was not possible with adult horse, but young animals were shown to be susceptible; two out of three of the inoculated 1-year-old foals died of encephalomyelitis with clinical signs and histopathologic lesions similar to those observed on the animal from which the strain had been obtained.

REPORT FROM THE VIROLOGY DEPARTMENT, PRINS LEOPOLD INSTITUUT VOOR TROPISCHE GENEESKUNDE, ANTWERPEN, BELGIUM

The effect of the addition of dextran to agar and agarose (Soc. Biol. Française) overlays on the plaqueforming of group A arboviruses and small and large plaqueformers of Middelburg (MB), Sindbis (Si) and WEE was completed. At the same time the usefulness of carboxy-methyl-cellulose as solidifying substance was studied. CMC was used at 0.9% final concentration. Group A arboviruses behave differently in respect to optimal plaquediameters under the conditions tested and can be grouped in:

1. Viruses for which DEAE addition to agar or agarose is not beneficial: Getah, SF, MBl, Sil, WEEl. Getah virus produces even better plaques under agar than under agarose.

2. Viruses for which DEAE addition to agar is beneficial in terms of plaque distinctiveness: Bebaru, Chik, EEE, Mayaro. The best overall useful concentration appears to be 62 γ/ml. in agar and 15γ in agarose.

3. Small plaque variants can produce large plaques under agar after the addition of DEAE but the minimal necessary concentration differs from 62γ for Sindbis-s to 125γ for WEEs to 250γ for MBs. Under agarose small plaques increase considerably in size, but only in the case of WEEs is agarose on itself sufficient; for MBs to produce large plaques under agarose DEAE must still be added. We observed no plaques under CMC overlay with Bebaru; Chik gave better results under agar than CMC.

Plaques were sharp and clear under CMC with EEE, Getah, Mayaro, Semliki F, Uzuma.

Large plaque variants of MB, Si and WEE produced smaller plaques under CMC than under agar, DEAE addition in this case did not alter plaque diameters.

Lowering the concentration of CMC increased plaque size, but the viscosity of the overlay rapidly becomes too low to obtain plaques. CMC overlay is very easy to handle since it requires no heating and can be delivered with an automatic syringe.

Aedes aegypti (Lagos strain) mosquitoes were experimentally infected with SF virus by feeding through a baudruche membrane on virus suspensions containing ATP. Virus titrations were performed in CETC with the plaque technique.

The results of titration of the mosquitoes immediately after feeding on different concentrations of virus gave the following results:

Virus titer in meal PFU/ml	Mosquitoes Positive	Mosquitoes Negative
10^3	-	4
10^4	2	9
10^5	4	7
10^6	4	2
10^7	9	-
10^8	8	-

The volume ingested by the mosquitoes varied from 10^{-3} to 10^{-5} ml.

The virus content of mosquitoes was determined after 1, 2, 3 and 4 weeks. After meals containing 10^7 and 10^8 PFU/ml. only 2/11 and 5/10 mosquitoes respectively contained virus at 28 days.

We hope to be able to compare the behaviour of this virus strain in other strains of A. aegypti, in view of the report from the South African Institute for Medical Research, Johannesburg in issue #14 of the Information Exchange.

Observations are currently under way to compare the behaviour of different plaqueforming strains of Middelburg, Sindbis, WEE viruses in mosquitoes.

We tested the sensitivity of the SIRC cell line (J. Leerhou, Arch. ges. virus, 1966, 19, 210-220) for some arboviruses. This cell line is currently used for the cultivation of rubella virus.

Only some group A viruses produced a CPE: EEE and chikungunya, other group A viruses tested produced only CPE when high titers were inoculated (Getah, Bebaru); of the group B viruses tested (WN, YF, RSSE and Jap B) only WN produced some CPE at dilutions 10^{-1} and 10^{-2}. Tahyna virus caused no CPE.

REPORT FROM LABORATOIRE DE MICROBIOLOGIE GÉNÉRALE ET MÉDICALE, SERVICE DE BACTÉRIOLOGIE ET PARASITOLOGIE, UNIVERSITÉ DE LIÈGE, BELGIQUE

The number of antigenic sites of Semliki Forest virus (SFV) was determined according to the method of Fazekas de St. Groth and Webster (1963). A preliminary report was published (Osterrieth, 1966).

To carry on these experiments, it was necessary to prepare concentrated gamma globulins. The methanol fractionation was found unsuitable because non-specific haemagglutinins present in the antiserum were concentrated together with the antibodies. Therefore another method was devised. The antiserum was centrifuged at 125,000 g for 18 hours to get rid of the lipids and lipoproteins. The bottom fraction, enriched in proteins, was fractionated by gel filtration on Sephadex G 200. The eluate contained three distinct protein peaks, one of which contained the non-specific haemagglutinins; another contained the antibodies. Fifty-eight % of the proteins of the antibody containing peak were gamma globulins. The details of this technique will appear in Acta Virologica.

To determine the number of antigenic sites of SFV it was necessary to determine the percentage of the antibodies of the gamma globulin preparation that was absorbed by various quantities of the virus. This was estimated by titrating the gamma globulin preparation before and

after absorption. Both the haemagglutination inhibition test and the neutralization tests were used for these titrations. As the results of both types of titrations were identical, the calculation (Fazekas de St. Groth and Webster, 1963) gave the same value for the number of sites capable of binding haemagglutination inhibiting antibodies and for the number of sites capable of binding neutralizing antibodies. One possible interpretation of these results is that there is only one kind of antibodies for both haemagglutination inhibition and neutralization of infectivity, and one kind of binding sites for these antibodies. As the sites for haemagglutination of the virus can be destroyed without impairing the infectivity (Osterrieth and Calberg, 1966) and thus are probably different, it is tentatively assumed that the inhibition of haemagglutination and the neutralization of infectivity by the antibodies is the result of steric hindrance.

The ability of SFV to fix complement in presence of antibodies is under study. It appears that caseinase C treated virus fixes less complement than untreated virus. Caseinase C treated virus fixes also less neutralizing antibodies. (Paul M. Osterrieth)

References: Fazekas de St. Groth, S. and Webster, R.G. (1963): J. Immunol. 90, 151.

Osterrieth, P. J. (1966): Life Sciences 5, 1825.

Osterrieth, P.M. and Calberg-Bacq, C. M. (1966): J. Gen. Microbiol. 43, 19.

REPORT FROM THE DEPARTMENT OF MEDICAL MICROBIOLOGY, FREE UNIVERSITY, AMSTERDAM, NETHERLANDS

Since the last contribution from this laboratory in the issue of March, 1964 the serological survey of some 150 human sera collected from 2 autochthonous population-groups in Surinam to study the presence of antibody and the relative frequency of antibody against 8 arboviruses belonging to group A, 5 viruses of group B, 4 viruses of group C, 3 of Bunyamwera, 2 of the Guamagroup as well as 4 yet ungrouped arboviruses, has been completed in the course of 1966. For the greater part results have already been published elsewhere, and the last will be submitted for publication before long.

Ornithological program: During the last years with varying intermittences sera have been collected from birds either migrating through or overwintering in this country. This autumn (1966) a bird-netting-bleeding-banding-releasing program was again set up in order to collect serum samples from a larger number of species of migratory birds passing through the Netherlands. The bird-species chosen were those which could be expected to have been reared during the spring-summer period in either southern Sweden, Finland or the Russian Taiga. Sera of 327 Turdidae (Field-fare, Redwing), Fringillidae (Longwinged finch, brambling) and Sturnidae ("Siberian" starlings) have been collected in the fall of 1966 in order to test these samples for CEE antibody in neutralization tests. We hope to be able to collect still some 200 sera from field-fares and redwings and wild geese (White -fronted goose and Beangoose) each during the overwintering period of these birds in this country.

It is anticipated that the present survey may serve as an indicator of the importance of certain bird species in the circulation of RSSE virus in nature. In order to gain a clearer idea of the course and the duration of a viraemia as well as the production and longevity of neutralizing antibody in certain bird species, wild ducks and wild pheasants have been infected with different doses of CEE virus (Graz I strain). It is hoped to perform similar experiments in Turdidae in case these birds survive long enough in captivity to make a follow-up study.

In collaboration with the Department of Veterinary Parasitology of the State University at Utrecht during the months of August and the beginning of September birds have been trapped in order to make a preliminary study of ticks infested on birds, breeding in a small area in the dunes of south Holland (not brought into cultivation). Ixodes ricinus larvae and/or nymphs were found on 9 dunnocks (Prunella modularis), 2 White throats (Sylvia communis), 3 Reedwarblers (Acrocephalus scirpaceus), 2 Willow warblers (Phylloscopus trochilus), 1 treesparrow (Passer montanus), 1 great tit (Parus major), 1 robin (Erithacus rubecula), 1 redstart (Phoenicurus phoenicurus), 1 blackbird (Turdus merula) 1 garden warbler (Sylvia borin), 1 starling (Sturnus vulgaris), 1 Herring Gull (Larus argentatus). A not yet identified Ixodes species was found on a linnet (Carduelis cannabina).

No virus isolations were performed.

REPORT FROM THE LABORATORY OF NEUROTROPIC VIRUSES,
INSTITUTE OF GENERAL PATHOLOGY,
UNIVERSITY OF AARHUS, AARHUS, DENMARK

Observations on West Nile Virus Infection in Mice:

The course of infection and the production of interferon was studied in mice following inoculation of West Nile virus by various routes.

Four-week-old Swiss white mice were inoculated intracerebrally, intravenously, intraperitoneally and subcutaneously with strain Egypt 101, using doses of 3-8 LD_{50} as tested by intracerebral inoculation into suckling mice. This dose resulted in a mortality rate of 100% in the adult mice when inoculated by the intracerebral route, as opposed to no mortality at all in the groups receiving subcutaneous injections. The mortality rates following inoculation by the intravenous and intraperitoneal routes ranged from 20 to 80%.

Groups of 10 mice each were sacrificed daily. The animals were bled from the jugular vein under ether anesthesia and the blood was pooled for each group. Pools of spleens and brains were ground in mortars with diluent (PBS, pH 7.4) to make up 10% tissue suspensions that were subsequently clarified by centrifugation.

Virus titrations were performed by intracerebral inoculation of suckling mice. Interferon assay was carried out - after dialysis at pH 2 and restoration of the pH to 7.4 - by the 50% plaque reduction method using mouse embryonic cells and Semliki Forest Disease virus as the test strain. In the case of the serum specimens dialysis was performed with material diluted 1:10.

The virus and interferon titers demonstrated in spleen, serum, and brain following intracerebral inoculation are shown in Figs. 1-3. As expected, an early and rapidly increasing production of virus was found in the brain; the titer was maintained at a high level during the last 2 to 3 days before death that occurred on the 5th to 6th day postinoculation (100% mortality). No interferon was detected on the first day postinoculation, after which there was a rapid increase, however, to fairly high concentrations that attained a maximum during the last 2-3 days. Spleen and serum were devoid of demonstrable amounts of virus on the first day postinoculation; a constant level was reached within the next few days. Only low titers of interferon were elicited in spleen and serum by this route of infection.

Intravenous and intraperitoneal inoculation resulted in essentially identical courses of infection. The virus and interferon titers obtained in spleen, serum and brain by intraperitoneal inoculation are shown in Figs. 4-6. No virus could be demonstrated in the brains until 3-4 days postinoculation when a rapid increase occurred, finally reaching almost the same titer as obtained by the intracerebral inoculation. In contrast, the mortality did not exceed 20-80% in these groups. Only moderate concentrations of interferon were found in the brain in the present experiment, though higher titers were obtained in other similar experiments. Early and fairly high interferon titers were found in the spleen, particularly when compared with the corresponding virus titers. Rather high titers of both virus and interferon were seen in the serum. The last few days of the infection were characterized by a marked decrease of virus and interferon in spleen and serum.

Inoculation by the subcutaneous route resulted in the development of mere traces of virus and interferon in serum on the 7th day postinoculation; at the same time traces of interferon were found in the spleen As mentioned above, all animals in this group survived the infection.

Further studies are in progress to elucidate the significance of the preliminary results presented here. (S. Haahr, E.A. Freundt)

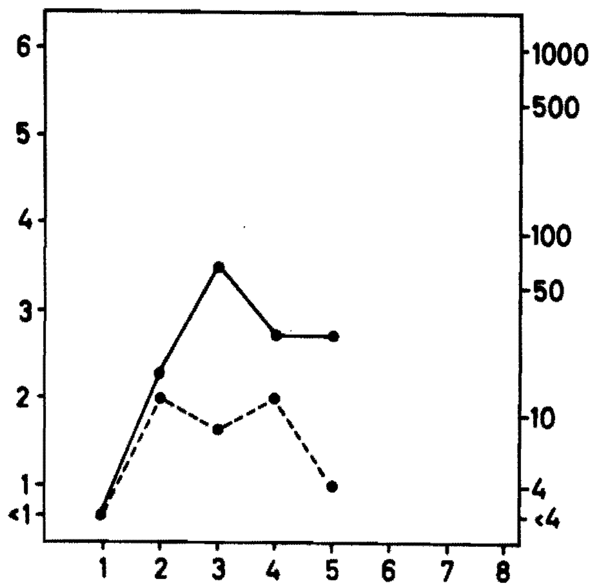


Fig. 1

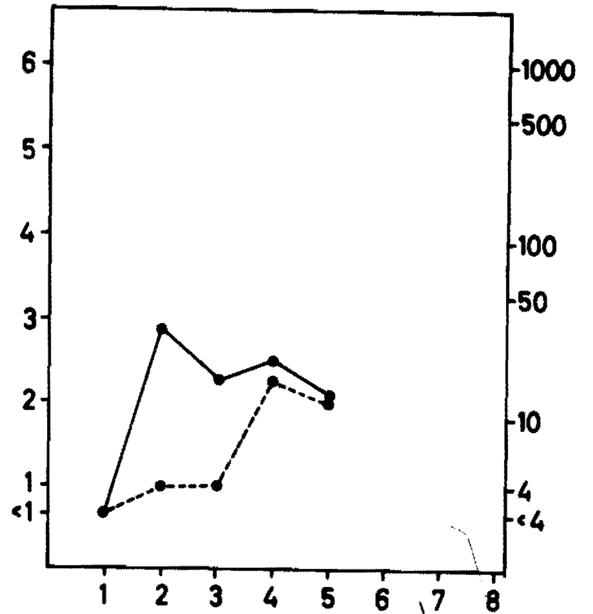


Fig. 2

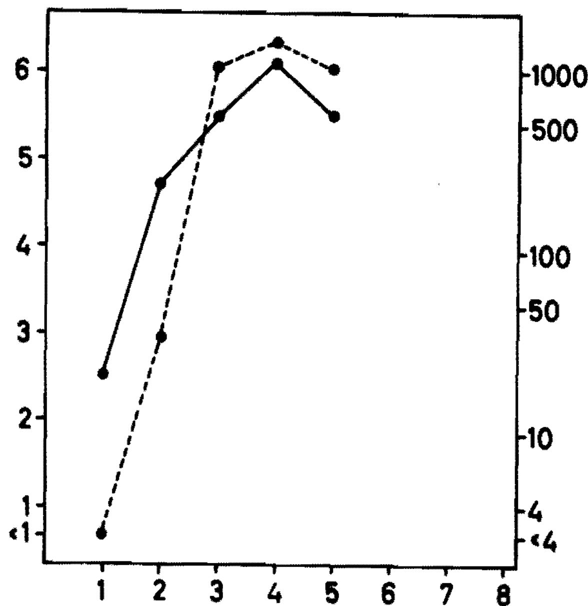


Fig. 3

Virus titer (•———•) and interferon titer (•-----•) in spleen (fig. 1), serum (fig. 2) and brain (fig. 3) of mice inoculated intracerebrally with West Nile virus. Abscissa: days after inoculation. Left ordinate: virus titers $\log_{10}$ LD_{50}/10 mg. Right ordinate: interferon titers/2oo mg.

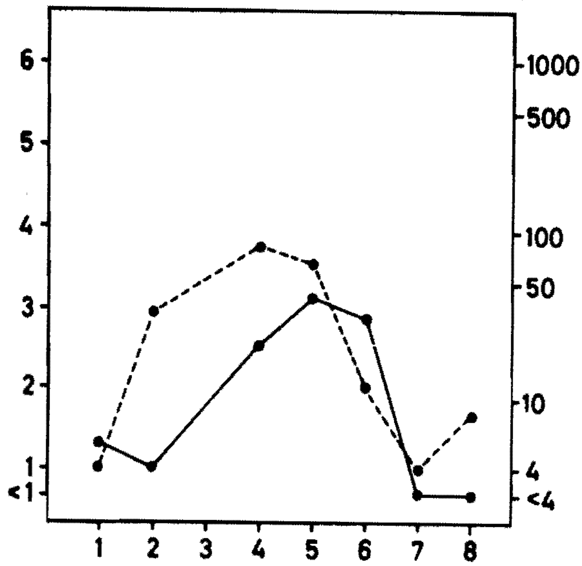


Fig. 4

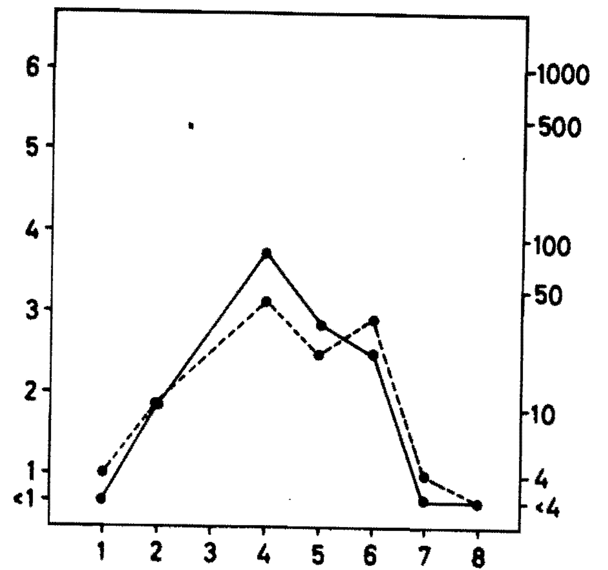


Fig. 5

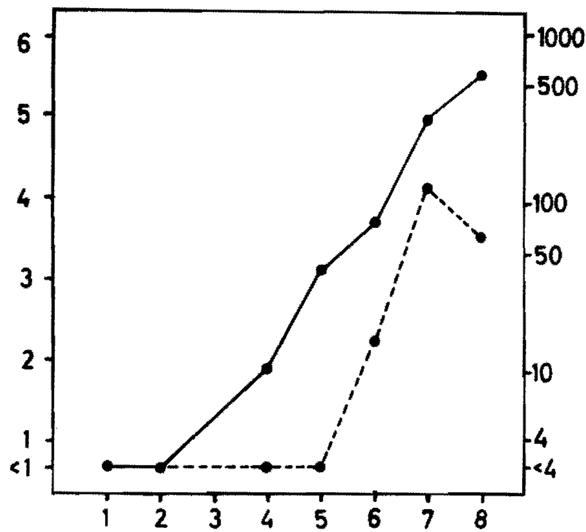


Fig. 6

Virus titer (•———•) and interferon titer (•-----•) in spleen (fig. 4), serum (fig. 5), and brain (fig. 6) of mice inoculated intraperitoneally with West Nile virus. Abscissa: days after inoculation. Left ordinate: virus titers $\log_{10}$ LD_{50}/10 mg. Right ordinate: interferon titers/200 mg.

REPORT FROM WHO REGIONAL REFERENCE LABORATORY FOR ARBOVIRUSES, BRATISLAVA, CZECHOSLOVAKIA

Study on Non-Specific Inhibitors to Ťahyňa and Čalovo Viruses with Respect to Other Arthropod-borne Viruses:

Our first problem with nonspecific inhibitors was encountered in 1964 when examing human sera from Košice region (Eastern Slovakia) in HI tests with various arbovirus antigens. Before shipment to the Institute, the sera were heated at 56° C. for 30 minutes. Thirty per cent of the examined sera reacted with Western equine (WEE), Eastern equine (EEE) encephalomyelitis viruses, with Semliki, Sindbis, West Nile (WN), tick-borne encephalitis (TBE) and Yellow fever (YF) antigens. Haemagglutinin inhibitor in the sera was probably activated by heating at 56° C. for 30 minutes. Therefore the present experiments were carried out.

For study on thermostable inhibitors of arboviruses animal sera have been selected. The thermostable inhibitor was detected in cow's sera only; in goat and chicken sera this type of inhibitor was not present. The thermostable inhibitor was detected after heating the sera at 56° C. for 30 minutes following kaolin extraction. The results of these experiments are presented in Table 1. It is evident that after heating at 56° C. the sera show an inhibitory activity against the haemagglutinins of all viruses tested. The most sensitive indicators of inhibitory activity of sera, as regards the highest titers detectable, was West Nile and Ťahyňa antigens.

In the present studies we try to throw a little light on the cross-group immunological reaction with the sera containing non-specific inhibitor. In all experiments we can discover cross-group reactions using 4-8 haemagglutinin units.

The action of nonspecific serum inhibitors on various HA units was studied using yellow fever antigen. In these experiments it was possible to demonstrate nonspecific inhibitors when using 8-16 HA units; with 32 or 64 HA units no positive reaction (1:20 or higher) was obtained.

Thermostable inhibitors in human and cow's sera were sensitive to delipidization; after ether treatment nonspecific inhibitors are removed.

In further experiments the effect of heating the sera at 100° C for 30

minutes before kaolin extraction was investigated. Table 2 indicates that heating at 100° C increases the inhibitory activity; the percentage of sera reacting with all antigens tested being 100 per cent.

Sera tested at 100° C had no inhibitory activity in virus neutralization tests. None of these sera showed any neutralization activity against TBE virus or WEE virus (Table 3). These experiments make it clear that inhibitors present after heating and kaolin treatment are non-specific in nature.

Studying the nature of non-specific inhibitors we try to investigate the effect of ether, $K10_4$ and trypsin. Delipidization of cow's serum by McFarlane's methods (1942) destroys non-specific inhibitors of serum. Trypsin did not destroy the inhibitory activity of the sera. After $K10_4$ treatment non-specific inhibitors disappeared, while decrease in HI titers of antibodies occurred.

In other experiments delipidized serum and lipoid fraction after ether and acetone treatment have been examined for the presence of inhibitors. It has been found that the lipoid phase contains non-specific inhibitors.

Finally the results on non-specific thermostable inhibitors in human and cow's sera show that inactivated sera should not be used in haemagglutination-inhibition tests with arthropod-borne viruses. (M. Grešíková and M. Szekeyová).

References: Grešíková, M., and Sekeyová, M. (1967): Non-specific Inhibitors of Arboviruses in Animal Sera. Acta Virol.: in press.

Verani, P. and Grešíková, M. (1966): Study on Non-specific Thermostable Inhibitors of Arboviruses in Human Sera. Acta Virol. 10: 421.

Table 1

Distribution of titre of inhibitors in cow's sera heated at $56^{\circ}C$ for 30' and kaolin extracted

Group	Antigen	Number of positives in titre					
		10	20	40	80	160	320
A	WEE	5	0	0	0	0	0
	EEE	8	8	0	0	0	0
B	TBE	9	1⌿	1⌿	1⌿	0	0
	WN	0	0	5	8	7	0
	YF	0	10	10	0	0	0
California	Ťahyňa	0	1	13	5	1	0
Bunyamwera	Čalovo	2	12	5	1	0	0

⌿ antibodies have been found in native sera

Table 2

Nature of inhibitory activity of human sera heated at 56°C and 100°C for 30 minutes.

Group	Antigen	Native and kaolin extracted sera	Heated at 56°C and kaolin extracted	Heated at 56°C and acetone extracted	Heated at 100°C and kaolin extracted	Heated at 100°C and acetone extracted
		Per cent of positive				
A	WEE	0	30	0	98	0
	EEE	0	49	0	98	0
B	TBE	3	43	10	96	0
	WN	0	75	0	98	0
	YF	0	54	0	98	0
California	Ťahyňa	20	71	62	98	0
Bunyamwera	Čalovo	0	40	0	97	0

Table 3

Testing of cow sera inactivated at 100 °C for 300 min. for the presence of nonspecific inhibitors in hemagglutination--inhibition and virus-neutralization tests.

	HI test after kaolin treatment							HI test after acetone treatment	Neutralization test
	WEE§	EEE	TBE	WN	YF	Ť	Č	WEE§ EEE TBE WN YF Ť Č	WEE§ TBE
1	40	40	40	80	80	80	40	No antibodies detected	No antibodies detected
2	40	40	40	80	80	80	40	No antibodies detected	No antibodies detected
3	40	80	160	160	320	80	80	No antibodies detected	No antibodies detected
4	40	40	20	80	160	40	40	No antibodies detected	No antibodies detected
5	40	40	40	80	160	80	40	No antibodies detected	No antibodies detected
6	40	40	20	40	80	80	20	No antibodies detected	No antibodies detected
7	40	80	20	80	80	80	40	No antibodies detected	No antibodies detected
8	40	40	40	80	80	80	40	No antibodies detected	No antibodies detected
9	40	80	40	80	160	80	40	No antibodies detected	No antibodies detected
10	20	40	40	80	80	40	20	No antibodies detected	No antibodies detected
11	40	40	40	160	160	40	20	No antibodies detected	No antibodies detected
12	40	40	40	80	160	80	40	No antibodies detected	No antibodies detected
13	20	40	20	80	80	40	20	No antibodies detected	No antibodies detected
14	40	40	20	80	160	80	20	No antibodies detected	No antibodies detected
15	40	40	20	80	40	40	20	No antibodies detected	No antibodies detected
16	40	40	40	80	80	40	20	No antibodies detected	No antibodies detected
17	40	160	40	160	160	80	40	No antibodies detected	No antibodies detected
18	40	80	20	80	160	80	20	No antibodies detected	No antibodies detected
19	40	40	20	80	160	40	20	No antibodies detected	No antibodies detected
20	40	40	20	80	80	80	20	No antibodies detected	No antibodies detected

HI - hemagglutination-inhibition

§ - antigen

Ť - Ťahyňa

Č - Čalovo

Transmission of the TBE Virus Haemaphysalis inermis Ticks to the Roe Deer (Capreolus capreolus) by Females of Ixodes ricinus and Nymphs:

The experiments on transmission of the TBE virus by virophoric females of Ixodes ricinus and nymphs of Haemaphysalis inermis to the roe deer were carried out. The nymphs of Ixodes ricinus and the larvae of Haemaphysalis inermis were infected on white mice. The mice were inoculated with 0.1 ml. of mouse brain suspension infected by the J 13 - 1965 hedgehog strain of Central European TBE virus in 7th mouse passage. The 10% brain suspension had a virus titer of 10^8 i.c. mouse LD_{50}/0.03 ml. Virus titrations were done by i.c. inoculation of white mice weighing 6 - 8 g. with 0.03 ml. volumes.

The TBE virus in the blood of roe deer was found from 2nd to 10th day after tick bite and the titer of virus was $<10^1$ i.c. mouse LD_{50} per 0.03 ml. The virophoric period (feeding of previous stage + premolting period + prefeeding period) in female of Ixodes ricinus amounts to 170 days, the virophoric period in nymphs of Haemaphysalis inermis amounts to 85 days.

On the basis of serological survey and these results, we consider the roes as indicators of TBE foci in nature. (J. Nosek, O. Kožuch, E. Ernek and M. Lichard).

Reference: Nosek, J., Kožuch, O., Ernek, E., Lichard, M. (1967): Übertragung des Zeckenencephalitis Virus (TBE) Durch die Weibchen von Ixodes ricinus und Nymphen Haemaphysalis inermis auf die Rehkalber (Capreolus capreolus). Zbl. Bakt. Parasitenkd. I. Orig.: in press.

Serological Survey and Isolation of TBE Virus from the Blood of the Mole (Talpa europaea) in a Natural Focus of Infection:

Experimental infection of the European mole (Talpa europaea) after subcutaneous inoculation of the TBE virus and the transmission of this virus by nymphs of Ixodes ricinus to the mole has been elucidated.

Out of 46 blood and brain samples, 7 positive isolations of TBE virus were obtained in spring season parallely in suckling mice and CEC cultures. The isolation rate in the locality Jarok near Nitra 3:5, in

the locality Jelenec 3:13 and in the locality Topolčianky 1:22. The highest titer of virus in the blood was $10^{1.6}$ mouse LD_{50} per 0.03 ml. The neutralizing antibodies against TBE virus were found in 26 per cent of 38 trapped moles in summer season.

The serological survey at the beginning of summer 1965 in elementary focus Topolčianky shows a high incidence of virus neutralizing antibodies in moles (26 per cent) in contrast to the low antibody incidence in small rodents (4-8 per cent) reported by Nosek et al. (1962). Age analyses of examined mole population in this season show that the neutralizing antibodies are present in moles younger than 3 months and 1 year. The importance of the mole in the circulation of TBE virus in an elementary focus is probably during the whole of the year. The long radius of action (some hundreds of meters), high infestation with larvae and nymphs of Ixodes ricinus, high titer of viraemia provide for dissemination of virophoric ticks also beside the elementary foci. The mole is important in temporal and spatial activity of a permanent focus during the year and successive years.

References: Kožuch, O., Grulich, I., Nosek, J., (1966): Serological Survey and Isolation of TBE Virus from the Blood of the Mole (Talpa Europaea) in a Natural Focus. (in press).

REPORT FROM VIROLOGICAL DEPARTMENT, RESEARCH INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY, BRATISLAVA, CZECHOSLOVAKIA

The African green monkey kidney cells, line GMK AH-1 are demonstrated as highly susceptible to the cytopathic effect of viruses belonging to the California complex (California encephalitis virus, La Crosse, Lumbo, Melao, Trivittatus and Ťahyňa viruses). The virus titer values expressed in CPD_{50}/0.1 ml. were comparable with those obtained in i.c. tests in weanling mice in parallel titrations. The plaque forming ability of California complex viruses was studied in bottle cultures of GMK cells under agar overlay. All virus strains tested formed well defined plaques after 48 hours' incubation. The size of plaques (0.5 - 5 mm.) was increased under agar overlay with 600 g/ml. of protamine sulphate. The PFU titer values recorded on the 5th day corresponded

with the CPD_{50} titers in the tube cultures under liquid medium.

The susceptibility of different human diploid cell strains derived in this laboratory from human embryo muscles (RIEM 2), lungs (RIEM 3), and from the whole human embryo (RIEM 4) to infection with Ťahyňa virus was compared. All Ťahyňa virus strains investigated except the tissue culture adapted line of the 92 strain (92 CEC) exhibited low or no cytopathic activity, although the virus multiplied well in HDCS as was shown in growth curves of the virus. The tissue culture adapted line of the 92 strain produced regularly CPE in HDCS. No marked differences were found in the susceptibility to Ťahyňa virus between particular human diploid cell strains.

The adsorption ability of two lines ("neuroadapted" and "cell-culture adapted" line of the 92 strain of the Ťahyňa virus) on three cell systems (chick embryo cells, grivet monkey kidney cells and human diploid cells) and some mouse tissues (striated muscle, brain and lung) in vitro was compared. The virus was applied in approximately equal doses in both lines - 1000 LD_{50}/1 ml. of tissue homogenate or cell suspension. The mixture was incubated in serial experiments at 37° C for 1 hour and shaken continuously or intermittently at 10-minute intervals. After the end of adsorption the mixture was centrifuged for 15 minutes at 3000 rpm. For the detection of the amount of virus adsorbed the plaque method was used. Cell-culture adapted line of the virus showed a higher adsorption ability than the neuroadapted line in all systems investigated. This difference was most distinctly observed in the case of adsorption of the virus to chick embryo cells and lung tissue of the mouse. The highest adsorption capacity was observed with grivet monkey kidney cells and striated muscles of mouse, the lowest with the chick embryo cells and lung tissue of mouse.

The reproduction of La Crosse virus in experimental animals was studied by the fluorescent antibody technique and by the biological methods and compared with that of Ťahyňa virus. The mode of reproduction of La Crosse virus in suckling mice resembles closely the multiplication of the extraneural variant of Ťahyňa virus in these animals. Following subcutaneous inoculation of about 300 LD_{50} per suckling mouse (2-3 days old), the first viral antigen was detected as early as 24 hours after inoculation simultaneously in many nerve cells of the brain, spinal cord, intravertebral ganglia. Foci of specific fluorescence were found at this time in the myocard, skeleton muscles and in the wall of some vessels in the heart. In following intervals the intensity of fluorescence increased and only at the peak of infection (i.e. 72 hrs. a.i.) viral antigen was visualised in the nerve cells of the

plexus myentericus Auerbachi and in one focus in the cortex of the kidney. It was interesting that the smooth muscles of the alimentary tract showed no specific fluorescence. Titrations of infectious virus in examined organs were in accordance with the results obtained by the fluorescent antibody technique. Although the nervous cells in experiments with La Crosse virus were notably sustained, the most involved ones from the extraneural tissues - both in experiments with Ťahyňa and La Crosse viruses - were striated muscles.

To elucidate the type of Ťahyňa virus viremia, the distribution of the virus in the blood of experimentally infected golden hamsters was studied. The presence of virus in the whole blood, plasma, cell elements and/or leucocytes obtained by separation procedure using the phytohemagglutinin was investigated, employing the conventional titration method in mice and also the fluorescent antibody technique in green monkey cell culture. Repeated experiments demonstrated that about 70 per cent of Ťahyňa virus in viremic hamster blood was associated with plasma. From the blood cell components the leucocytes were found to be not a significant vehicle of Ťahyňa virus.

During summer months in selected southern Slovak area with mass evidence of mosquitoes 80 paired sera from children with acute febrile illness were collected. In six patients significant rise of antibody titers against Ťahyňa virus in the virus neutralization, and/or complement fixation and hemagglutination inhibition tests were detected. Arbovirus A, B and Bunyamwera group, influenza A, B and adenovirus infections were excluded. In all cases a mild course of illness lasting four to eight days was observed, characterized by fever up to 39° C., pharyngitis and tonsillitis. Occasionally dizziness was also observed. The virus isolation experiments from the blood in the acute phase of the illness by tissue culture technique were not successful.

(Bárdoš, V., Šimková, A., Šefčovičová, L., Čupková, E., Wallnerová, Z., Schwanzerová, I., Schwanzer, V.)

REPORT FROM THE INSTITUTE OF MICROBIOLOGY, UNIVERSITY OF PARMA, ITALY

Complete and Coreless Hemagglutinating Particles of Group A Arbovirus (Sindbis):

During 1966, the complete and coreless hemagglutinating particles of Sindbis virus have been studied.

According to previous observations by Mussgay and Rott (1964) and also by us (1965), the CsCl equilibrium density gradient centrifugation has demonstrated two hemagglutinating components: one more dense, coinciding with the peak of infectivity; the other, less dense, with a lower infectivity-hemagglutination ratio.

The percentage of hemagglutinating activity in two peaks varied in each preparation.

The isolation and recentrifugation of each hemagglutinating component gave only one peak in the same approximate position of the original peak. The same results have been obtained also when the denser and less dense components of different preparations were pooled and recentrifugated. In the cases in which dense peaks presented some degree of asymmetry, the CsCl density gradient recentrifugation - according to observations on dengue 2 virus (Stevens and Schlesinger) - gave sometimes two peaks in the same position, respectively, observed for the more and less dense original peaks.

According to Mussgay and Rott, the electron microscopic examination of the denser component revealed spherical particles 60-70 mμ. in diameter with a spherical inner core (complete virus). The less dense component presented pleomorphic forms, without internal structure (incomplete virus).

Although more stable than the analogous particles of ECHO 12 virus (Halperen et al., 1964), the empty forms of Sindbis virus are less stable at 37° C. than the homologous full forms.

In another group of experiments, we have studied the relation between the hemagglutinating particles demonstrated in the virus subjected to density gradient centrifugation and the hemagglutinating components observed by means of column chromatography (Gordon Smitn and Holt, 1961). According to our previous experiments, the hemagglutinating

activity of Sindbis virus was eluted over the whole range of molarities employed, but maximally at 0.1 and 0.2 M. The lowest infectivity-hemagglutination ratios were frequently found - but not always - at the lowest molarities. Generally the hemagglutinating activity was not completely sedimented at 40.000 r.p.m. per 60', but - with the exception of one experiment - it was sedimented after a new centrifugation at the same speed and time.

On the whole, the results of our researches have not demonstrated any relation between the chromatographic fractions and the hemagglutinating components evidenced after density gradient centrifugation:

a) both the low and high molarity fractions revealed, after density gradient centrifugation, two hemagglutinating peaks, respectively, more and less dense;
b) both "full" and "empty" hemagglutinating components were eluted over the whole range of molarities employed;
c) the electron microscopic examination of low and high molarity fractions did not reveal particular differences.

In another group of experiments, we have studied the kinetics of production of two types of hemagglutinating particles and their behavior in cellular cultures subjected to prolonged incubation.

The results obtained demonstrated that both types of particles are formed at approximately parallel rates. It is in agreement with analogous results obtained for the C and D antigens of poliovirus (Scharff and Levintow, 1963), and for the complete and coreless hemagglutinating particles of ECHO 12 virus (Halperen et al., 1964).

On the other hand, we observed that in the cellular cultures subjected to prolonged incubation (72 h) the hemagglutinating activity was principally associated with the less dense peak (76.1%), while in those subjected to more brief periods of incubation (24 h) it was found mainly in correspondence of the denser peak (71.9%). On the contrary, the infectivity was, after 72 h of incubation, exclusively localized in the denser peak, while the less dense fractions were completely non-infectious. These last results seem to indicate that, besides the empty spontaneous forms, other coreless particles, without RNA for thermic degradation of complete virions, can be produced.

Serological Survey. As we previously related, we collected a high number of human sera from different parts of the Po Valley (Parma, Piacenza, Mantova). These sera have been examined against a number

of different arboviruses: Group A: Sindbis (HAI); Group B: West Nile, Ntaya, Langat (HAI); Group C: Tahyna (NT).

We are now collecting a considerable number of sera from Sardinia and from some departments of the Mediterranean coast: La Spezia and Imperia. At La Spezia, we found three cases of human encephalitis with a significant rise of antibodies against West Nile; Sardinia and Imperia are near, respectively, to Corsica and to the border of the south of France, where Hannoun, Pantier and Corniou found a high percentage of antibodies against West Nile or Group B viruses.

Up to now, a relatively small number of sera collected from Sardinia, La Spezia and Imperia have been tested, but the serological survey will be extended in 1967. The results obtained are summarized in Table 1.

Symposium on Arboviruses. In coincidence with the 13th Congress of the Italian Society for Microbiology, held in Parma-Salsomaggiore from 3rd-5th May 1965, a Symposium on Arboviruses was organized by the Institute of Microbiology of the University of Parma.

The following researchers gave a lecture on the following subjects:

W. McD. Hammon (Chairman): General properties and classification of arboviruses;
A. Sanna: The hemagglutination by arboviruses;
J.S. Porterfield: The serological relationship among arboviruses;
M. Mussgay: Isolation and synthesis of substructures of arboviruses;
G. Rita et al.: Interference and interferon among arboviruses;
A.C. Saenz: The importance of arbovirus infections as a cause of human disease: a W.H.O. programme in this field;
V. Bardos: On the medical importance of the two Czechoslovak mosquito-borne arboviruses Tahyna and Calovo,

The reports of the Symposium can be requested from: Instituto di Microbiologia dell'Universita - Ospedale Maggiore - Parma, Italy.

(A. Sanna)

Area	Sindbis HAI			West Nile HAI			Ntaya HAI			Langat HAI			Tahyna NT		
	tested N.	positive N.	%	tested N.	positive N.	%	tested N.	positive N.	%	tested N.	positive N.	%	tested N.	positive N.	%
Po Valley 1962	500	17	3.4	500	62	12.4	500	47	9.4	-	-	-	-	-	
1965-66	659	16	2.4	659	35	5.3	659	70	10.6	659	12	1.8	600	8	1.3
	1159	33	2.8	1159	97	8.3	1159	117	10.09	659	12	1.8	600	8	1.3
Sardinia (Sassari)	310	-	-	310	29	9.3	310	31	10	310	8	2.5	183	3	1.6
La Spezia	217	4	1.8	217	28	12.9	217	70	32.2	217	14	6.4	-	-	-
Imperia	145	-	-	145	12	8.2	145	29	20	145	-	-	-	-	-

TABLE I - Antibodies against Arboviruses in human sera from different parts of Italy.

REPORT FROM ARBOVIRUS LABORATORY, PASTEUR INSTITUTE, DAKAR, SENEGAL

Yellow Fever Outbreak in Senegal.

Retrospective inquiry revealed that the epidemic occurred in July 1965, at the beginning of the rainy season and ended in December, some sporadic cases occuring till mid-January 1966. The deaths by week are shown in Figure 1A. It was estimated that not less than 2,000 cases (between 2,000 and 20,000 according to the inquirers) had occurred with a case fatality rate of 16%. 90% of the patients were under 10 years of age (Figure 1B) the older being immunized by previous mass vaccination campaign with the Dakar scratch vaccine.

The epidemic stopped at the frontiers of Diourbel and Mbacké Departments. This area was that of maximum Aedes (Stegomyia) aegypti population in consequence of local custom in collecting water for domestic purposes. The water is kept in earthen tanks, half buried in the ground so that they are never completely emptied and serve as common breeding places for Aedes (Stegomyia) aegypti.

The area involved, 3,000 square kilometers, 140 km. east of Dakar, is an arboreous savannah, at altitude under 100 meters. Climate is of the Sudanese type, very dry with a rainy season from mid-July to mid-October. The population, about 140,000 with 50,000 under ten years of age, is distributed among small agricultural and cattle raising communities. Yearly, between July and December, 30,000 laborers walk from Portuguese Guinea to the Diourbel region to work on earthnut cultivation.

The first information came from clinical and pathological diagnosis. The clinical picture was as usual: mild fever, high fever with one or more of the classic symptoms: nausea, vomiting, jaundice, albuminuria and typical hepatonephritis. The differential diagnosis was complicated by hyperendemic malaria and an infectious hepatitis epidemic occuring at the same time. Liver biopsy and necropsy specimens were obtained from 60 patients and 46 were found to show changes typical of yellow fever.

Attempts to isolate virus from human blood samples were successful. 16 strains were obtained from blood taken 1 to 7 days after the onset. Reisolation was successful in the 6 available specimens processed.

On first passage into mice, the incubation period was 8-13 days. The identification was made by HI, CF and NT cross-reactions. These strains are antigenically very closed of our yellow fever reference strain (FNV).

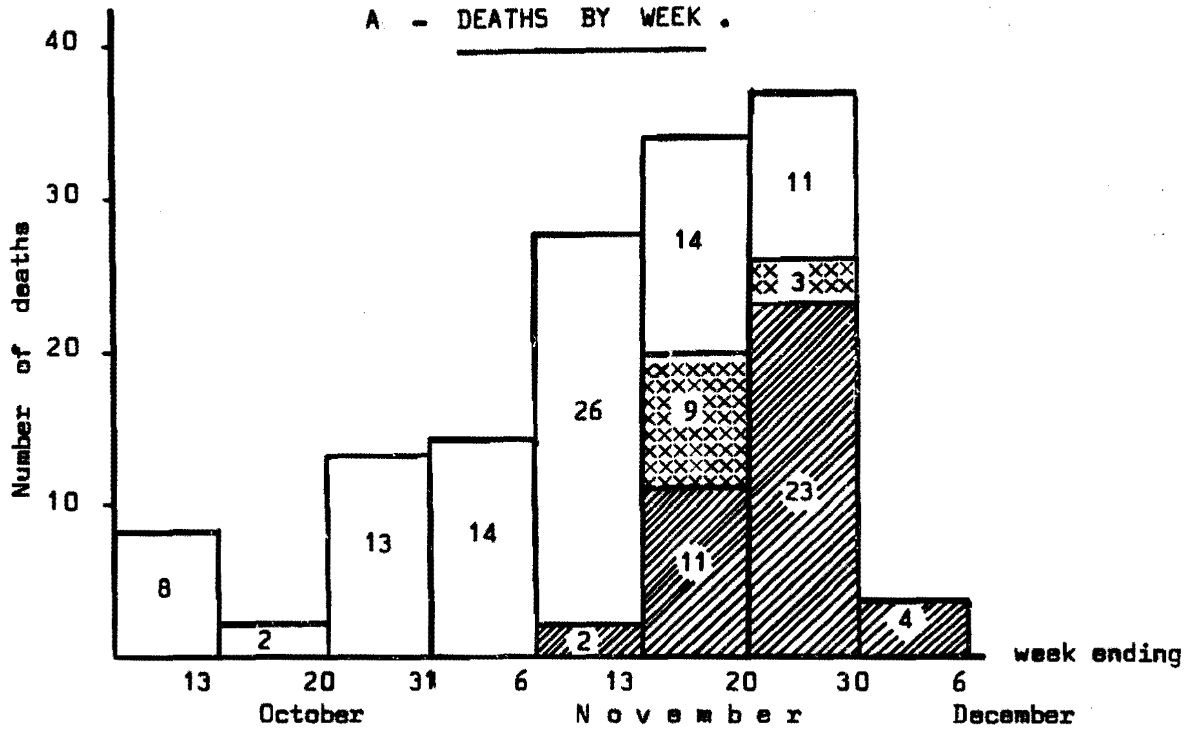
Of 38 paired sera taken in the epidemic area, 19 showed clear-cut conversion for yellow fever antigen in HI and CF tests.

It is unknown whether the yellow fever virus was imported into the area by laborers from Portuguese Guinea or whether the outbreak began in the Diourbel region itself. Serological surveys in the epidemic zone showed that 44% of children under 10 years had HI antibodies against yellow fever. In the peripheric zone, of 930 sera tested in the same age-group, 5% only have HI antibodies against yellow fever antigen. But we found some evidence of yellow fever virus activity with positive CF test which may be indicative of recent infection, peculiarly in Casamance along the frontier area.

A program of captures of insects and vertebrates was carried out. As a consequence of intensive insect control operation by the spraying of insecticides, only few mosquitoes were available for virus isolation. No virus was recovered. Numerous vertebrate species were shot or trapped and their organs and sera inoculated into mice and tested for antibodies by CF, HI and protection test. Complete results will be published very soon. Most interesting are serological results in 75 monkeys shot or caught in different parts of Senegal, Casamance and Gambia. Results obtained are summarized in Table 1. 30% were positive for yellow fever antibodies. Among the 23 positive, 8 (34.7%) had positive CF test which may be indicative of recent infection.

An extensive vaccination campaign was carried out in order to prevent recrudescence and possible spread to neighbouring areas. As shown in Table 2, more than 2,000,000 people were vaccinated in the infected area, in a barrier area surrounding it and in principal towns.

FIGURE 1



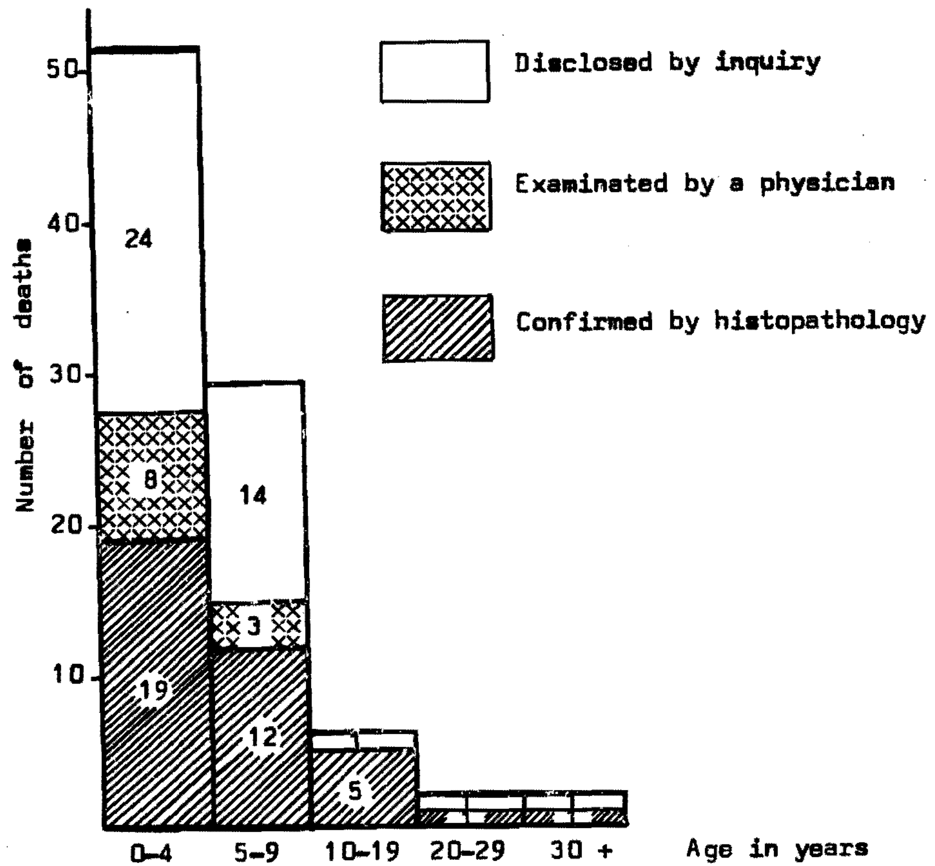


TABLE 1

RESULTS OF YELLOW FEVER ANTIBODIES STUDIES ON 75 MONKEYS

SPECIES	EXAMINATED	POSITIVE	%	HI	CF	NT
Cercopithecus aethiops	30	9	30	6	3 (1)	9/9 (2)
Erythrocebus patas	22	7	31	7	3	3/6
Papio papio	8	4	50	4	1	1/3
Colobus badius	15	3	20	3	1	1/2
TOTAL	75	23	30	20	8	14/20

(1) 2 anticomplementary sera - (2) Positive/tested

CORRELATION BETWEEN HI, CF, and NT TESTS

Number of sera	HI	CF	NT
6	+	+	+
2	+	+	ND (1)
2	+	ac	+
3	+	0 (2)	+
7	+	0	0
3	0	0	+

(1)Insufficient quantity of serum

(2) 0 = less than 1/20 in HI, less than 1/8 in CF and less than 75% of animals survive in NT

TABLE 2

RESULTS OF MASS VACCINATION CAMPAIGN

Locality	Type of vaccine		Total
	Dakar Strain	17 D	
Diourbel	505,500	43,674	549,174
Cap Vert et Dakar	498,500	67,500	566,000
Sine Saloum	379,500	32,235	411,735
Thies	266,500	1,500	268,000
Casamance	50,000*	9,800	59,800
Région du Fleuve	149,000	9,500	158,500
Sénégal Oriental	20,000	-	20,000
Total	1,869,000	164,209	2,033,209

* approximately.

REPORT FROM THE ARBOVIRUS RESEARCH PROGRAM, UNIVERSITY OF IBADAN, NIGERIA

The isolation of two Simbu group viruses from cattle blood was recorded in 1965 from the Ibadan laboratory. These were AN5077 and AN5550. Six additional isolations of related agents were obtained in 1966 from goat (AN9398), cattle (AN10598, AN10599, AN10107), sheep (AN11893) and human (H11003) blood. Virus AN5077 was compared with other Simbu group isolates at the Yale Arbovirus Research Unit (YARU) and at the East African Virus Research Institute and found to differ from all Simbu group strains tested. At YARU AN5550 was shown to be indistinguishable from Sathuperi by CF testing.

In the Ibadan laboratory neutralization testing of the eight local isolates has shown them to represent 4 distinct serological types. Two previous Nigerian Simbu group isolates (Yaba 1 and Yaba 7) obtained from mosquitoes in 1962-63 at the West African Council for Medical Research Laboratory (WACMR) also were compared in NT and shown to differ from each other and from the 4 Ibadan types. Thus, among 10 Simbu group isolates obtained over a period of five years in Nigeria there appear to be 6 distinct serological types represented (Table 1). Recognition of this diversity in type is important when serological surveys are made.

Limited attempts have been made to prepare hemagglutinating antigen from these strains. The only successful antigen in Ibadan was that obtained by Miss Amelia Andrade (visiting serologist from Belem) in 1965 from AN5077 by the sucrose acetone and protamine sulphate technique, with optimum pH 5.75 at 37°C. This was used in a serological survey on human and domestic animal blood. No HAI antibodies for this virus were detected in human sera. At 1:20 dilution with 4 units antigen cattle sera showed HAI antibodies for AN5077 in 20 of 275 samples, sheep in 2 of 32, goat in 5 of 49, and swine in one of 32.

A flock of sheep from which an isolate (AN11893) identical with AN10107 was obtained showed 45% with NT antibodies when the virus was isolated in October 1966. Ten months previously the antibody rate for this virus had been 27%. Of the 16 pairs of sera available for comparison one showed conversion from negative to positive for antibodies to this virus, seven were positive in both samples and eight were negative in both samples. Further NT with Simbu group viruses and human and domestic animal sera are in progress. (Dr. Ottis R. Causey)

Table 1

COMPARISON OF SIX SIMBU GROUP AGENTS FROM NIGERIA

BY NEUTRALIZATION TESTING

Antigen	Log LD_{50}	ANTISERUM Neutralization indices 5077	5550	9398	10107	Yaba 1	Yaba 7
AN5077	6.5	$\geq$ 4.3	0	0	$\leq$0.5	$\leq$0.5	0.6
AN5550	7.4	1.4	4.4	1.4	0.4	0.6	0.5
AN9398	6.7	1.1	2.2	$\geq$5.0	0.2	0	0
AN10107	6.9	1.0	1.2	$\leq$ 0.7	$\geq$5.0	$\leq$1.1	$\leq$1.9
Yaba 1	6.6	$\leq$1.1	$\leq$1.1	$\leq$1.2	$\leq$1.3	2.5	1.1
Yaba 7	7.4	$\leq$0.9	$\leq$2.0	$\leq$1.9	$\leq$1.9	1.9	4.4

REPORT FROM THE VIROLOGICAL SECTION OF THE DUTCH MEDICAL RESEARCH CENTRE, NAIROBI, KENYA

The two virus strains, isolated from Aedes pembaensis near Mombasa and mentioned in the issue of September 1966, have been identified at the East African Virus Research Institute as closely related to or identical with Lumbo virus.

From 14th November till 19th December 1966, during and shortly after the short rainy season, a virus isolation experiment was conducted near Malindi, 75 miles north of Mombasa, in the coastal area of Kenya. A total of 23001 mosquitoes were collected, mainly on human baits, identified, pooled according to species and inoculated in baby mice. Three shipments of pregnant mice, received at regular intervals at the field laboratory, provided the litters. A total of 27 species or groups of related species were represented among the collections. Of 14 species sufficient specimens were collected to be suspended and inoculated. So far six virus strains have been isolated and sent to the East African Virus Research Institute for identification. We are not yet sure about a seventh agent, which makes mice ill after a long incubation period and kills after a long average survival time.

The table summarizes the results of the experiment.

Mosquito species	Number collected	Number of pools	Number of isolations
Mansonia (Mansonioides) africana	1967	16	0
- - uniformis	17901	43	2 or 3
Aedes (Mucidus) scatophagoides	26	2	1
- (Neomelaniconion) albicosta	278	4	0
- (Skusea) pembaensis	121	3	0
Culex (Culex) poicilipes	432	7	2
- - sitiens	54	2	0
- - thalassius-tritaeniorhynchus group	164	4	1
- - antennatus	39	2	0
Anopheles (Anopheles) coustani tenebrosis	1779	10	0
- - - ziemanni	18	1	0
- (Cellia) pharoensis	34	1	0
- - gambiae	28	2	0
- - funestus	120	2	0
Total 14 species / groups	22961	99	6 or 7

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

Field studies extending over 11 years were completed in 1966 in Tongaland, northern Natal province, South Africa. Smithburn et al. (1957) reported on work carried out in 1955 and Worth et al. (1961) dealt with the years from 1956 to 1960. It is our intention here to comment briefly on some aspects of the entire period, viz., from April 1955 through to February 1966.

Tongaland is in the coastal lowlands on the eastern seaboard, bordering on Mocambique. On the basis of temperature it is classified as tropical, but its rainfall is only moderate; 28 inches, often less, with 75% occurring during 5 months of the year. From 1956 studies were confined to an area near to the confluence of the Usutu and Pongola Rivers. In this area mosquito breeding is partly independent of local rainfall because of flooding of low-lying ground by these two rivers as the result of rain further inland. The study area is therefore more favorable for viruses than Tongaland as a whole.

Mosquitoes were usually collected during visits of about 10 days each, with 4 to 9 visits a year. During 1958 to 1960, however, visits were frequently of longer duration, the longest being of 65 days. Mosquitoes were pooled by species and tested for virus by IC inoculation of infant mice. Man, birds and rodents were also tested for virus.

A total of 294,169 mosquitoes was tested and this resulted in the isolation of 117 strains of arboviruses, representing at least 14 distinct types as some viruses of the Bunyamwera group were only identified to group level. A further 6 strains were isolated from man (4) and wild birds (2). Eighty strains (68%) belonging to 10 types were isolated from Aedes circumluteolus whereas only 37 strains came from all other species as shown in Table 1. There is little doubt that in Tongaland this mosquito is the main vector of Wesselsbron, Spondweni, Pongola and Bunyamwera viruses. This is suggested by the relatively higher frequency of isolations of these viruses from circumluteolus in comparison to those from other species (Table 1). In addition, Simbu, Rift Valley fever and AR3896 (Lebombo) viruses were only isolated from circumluteolus. This mosquito was one of the most abundant collected and comprised 54% of the total tested for virus.

In Table 2 the number of strains of each viral type are analysed

according to the summer season during which they were isolated. It will be seen that Pongola, Spondweni, Wesselsbron, Bunyamwera and Middelburg viruses, in that order, were the five most frequently isolated viruses. These viruses, furthermore, were isolated during a significant number of summers from which it would seem that they are permanently established within the somewhat limited area studied. Worth et al. (1961) drew attention to the sporadic nature of detectable viral activity by specific viruses in Tongaland. This aspect was more fully and convincingly dealt with by them than space here allows. This is also apparent from Table 2 even with those viruses frequently isolated. For example, Pongola virus was not isolated during 5 of the 11 summers and many other viruses show a similar tendency and no isolations were made in the 1964-65 summer.

While some of this intermittency is undoubtedly an artefact due to the impossibility of observing all places and all things all of the time, it is also possible that this might be the result of a real phenomenon. As suggested by Worth et al. (loc. cit.) the sporadic appearance of these viruses might reflect the existence of cryptic viral phases when virus is present in an area in an unusual form when it cannot be detected by conventional methods. This could mean that a mosquito-borne virus might survive in an area without the need for continuous mosquito transmission. A similar situation apparently occurs with Sindbis and West Nile viruses in the temperate, inland plateau region of South Africa where, on account of the inclement winters, all mosquito-borne transmission must definitely cease for 4 or 5 months of the year and yet these viruses appear regularly each summer without any evidence of introduction from tropical areas (McIntosh et al., in press). In this connection similar examples from other parts of the world come to mind.

Furthermore, the utilization of circumluteolus as maintenance vector raises an interesting point with regard to viral survival. Populations of this mosquito fall to very low levels during the dry season and it is doubtful whether numbers would be adequate to maintain cyclical transmission at this time. Nor does it seem likely that hardier species such as Culex univittatus could assume the role of maintenance vector during dry periods in view of the infrequency and, in the case of some viruses, even failure, to isolate them from these mosquitoes during the wet months. Hence, basically the same difficulty of explaining viral survival exists in Tongaland, a tropical habitat, as in the temperate regions of South Africa. In both regions a long period occurs when mosquito-borne transmission does not apparently take place.

If such cryptic phases of arboviruses did occur it could mean that even mosquito species with short periods of prevalence might well act as main vectors, since the need for continuous mosquito transmission would not arise. Such a possibility seems likely with regard to chikungunya virus in the study area of Tongaland. This virus has never been isolated there and yet 54 out of 94 vervet monkeys recently trapped in the area have chikungunya antibodies. Among the mosquitoes feeding on these monkeys in the canopy at night are members of the *Aedes furcifer/taylori* group, known efficient vectors of chikungunya virus, whereas the two most prevalent species feeding on these monkeys are either poor or non-vectors. Mosquito collecting methods in Tongaland have not favoured the collection of significant numbers of *furcifer* so that even had these mosquitoes been carrying chikungunya virus during the short periods when they existed as blood-feeding adults it is unlikely that virus would have been isolated from them. On account of their marked seasonal prevalence in this area one would normally tend to ignore the *furcifer* group as the main vectors infecting these monkeys. From what has been said this may be a misleading viewpoint. (B. M. McIntosh and P. G. Jupp)

References: McIntosh, et al. (in press): *S. Afr. J. Med. Sci.*
Smithburn, et al. (1957): *S. Afr. J. Med. Sci.* 22: 41-126.
Worth et al. (1961): *Am. J. Trop. Med. & Hyg.* 10: 583-592.

Table 1

Number of isolations of each viral type from Aedes circumluteolus compared with other mosquitoes in Tongaland, 1955/66

Group	A			B				Bun.	Bwamba	Simbu		Ungrouped			Total
Virus	Sindbis	Middelburg	Ndumu	Wesselsbron	Spondweni	W. Nile	Usutu	Bun. & others	Pongola	Simbu	Ingwavuma	Rift Valley F.	Mossuril	AR3896	
A.circum-luteolus	1	3	2	12	12	0	0	12	32	3	0	2	0	1	80
Other mosq.	6	7	1	3	8	1	1	4	3	0	1	0	2	0	37

Table 2

Number of strains of each virus isolated each summer season in Tongaland 1955/66

Virus	summer seasons 1955/56	56/57	57/58	58/59	59/60	60/61	61/62	62/63	63/64	64/65	65/66	Total
Sindbis				2	2		2	1				7
Middelburg		2		1	1		4	1			1	10
Ndumu				2		1						3
Wesselsbron	1 + 1*	2	3	5	2				2		1*	17
Spondweni	1		9	5	1		4					20
W. Nile			2*					1				3
Usutu				1								1
Bun.Group	1 + 1*	3		2	2		2		5		1	17
Pongola	10		2		9	1	2		11			35
Simbu	1		1						1			3
Ingwavuma					1*		1					2
Rift V. F.	2											2
Mossuril							1		1			2
AR3896						1						1
	18	7	17	18	18	3	16	3	20	0	3	123

* = isolations from non-mosquito source

NOTICE OF SPECIAL SERVICE

Mammal Identification Services Established:

In cooperation with the National Institute of Allergy and Infectious Diseases, National Institutes of Health, The Smithsonian Institution in Washington, D. C. has established a mammal identification service dedicated initially to servicing collections and specimens submitted by NIH laboratories.

The epidemiologic studies of research investigators of the Middle America Research Unit, the Rocky Mountain Laboratory, and other laboratories affiliated with the National Institutes of Health, frequently involve wild rodents and other mammals as possible carriers or reservoirs of etiologic agents of human diseases. The necessity for providing a firm taxonomic basis for field and laboratory studies of diseases involving wild mammals is obvious and imperative, and, of course, adequate study and report of the mammalian aspects of diseases requires positive and authoritative identification of the host specimens collected.

In most instances (when the specimens originate outside the U. S.) identification is a difficult process. The early descriptions of mammals often were based on a single specimen or even on hear-say evidence and often were published without reference to the work of other zoologists. This frequently resulted in a bewildering multiplicity of names for even the most common species. Now, collections from more remote regions in South America, Africa and Asia are becoming sufficiently extensive to permit a reasonable evaluation of the limits of morphological variation in species, to permit accurate definition of the species, and to permit application of the proper names. In addition to providing authoritative identification, this service will draw up identification manuals and field keys for areas under study by NIH scientists. To ensure the priority of this project the Smithsonian has assigned a scientist and a technician to it on a full-time basis.

Non-NIH agencies are welcome to use the identification service but will be charged on a per specimen or separate contract basis, and the Smithsonian will credit monies received to the cost of operating the service. For further information write to Dr. Gary L. Ranck, Assistant Curator, Division of Mammals, U. S. National Museum, Washington, D. C. 20560.

EPIDEMIOLOGICAL NOTES FROM WEEKLY EPIDEMIOLOGICAL REPORT OF THE PAN AMERICAN SANITARY BUREAU, REGIONAL OFFICE OF THE WORLD HEALTH ORGANIZATION, WASHINGTON, D.C.

Dengue in the Caribbean Region:

In 1966, to date, no outbreaks of dengue have been reported from the islands of the Caribbean. Three cases were reported in Jamaica in the early part of the year, and one case in Puerto Rico in the week ended 15 October. In Venezuela, 4,922 cases of dengue have been reported to 24 September. The weekly reported incidence has declined steadily since May, and only 389 of the cases correspond to the third quarter of the year. The States in which large numbers of cases have been diagnosed during 1966 are all in the western part of the country: Carabobo, Cojedes, Falcon, Guarico, Lara, Merida and Trujillo.

EDITORIAL NOTICE

This issue is the first produced under auspices of U.C.L.A., with continued reproduction and distribution by the Arbovirus Infections Unit of the National Communicable Disease Center. The shift and involvement of new personnel has resulted in some delay. The result reflects how well the new transcriber, Harriet Ziegler, has learned her role from Betty Foster.

The next issue is due out in October. Deadline for contributions is October first. They should be addressed to:

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