ECOLOGICAL INVESTIGATIONS PROGRAM
ARBOVIRAL DISEASE SECTION
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ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

63

Number Nine March 1964

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

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ANNOUNCEMENT FROM CHAIRMAN, EXECUTIVE COMMITTEE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

The next annual general meeting of scientists which the American Committee on Arthropod-borne Viruses represent will be held on Wednesday morning, November 4, 1964, prior to the 13th Annual Meeting of the American Society of Tropical Medicine and Hygiene, at the Waldorf-Astoria Hotel in New York. This meeting provides a forum for discussion of pertinent subjects and problems in arbovirus research. The ACAV began its activities in 1961 as representatives of American scientists interested in arboviruses; current membership includes Drs. Buescher, Downs, Hammon, Scherer, Shelokov, and Taylor, with Dr. Reeves serving as ex-officio treasurer. In accordance with existing policy, a member is replaced each year (Dr. Work in 1962, Dr. Reeves in 1963, and Dr. Taylor in 1964). At each annual general meeting, a new member is selected from nominees presented by a nominating committee and by others from the floor. The activities of the ACAV are carried out by subcommittees. Currently these include the Subcommittee on Information Exchange (Chairman Dr. Taylor), Subcommittee on Serologic Reagents (Chairman Dr. Casals), Subcommittee on Relationship of Birds to Arboviruses (Chairman Dr. Hayes) and a newly created Subcommittee on Laboratory Infections (Chairman Dr. Hanson). It is urgently requested that problems related to arbovirus research be brought to the attention of the ACAV. Without such suggestions, it is difficult to represent the community of scientists interested in arboviruses. Please make known topics warranting discussion at the next meeting by writing to Dr. W.F. Scherer, Dept. of Microbiology, Cornell University Medical College, 1300 York Ave., New York, N.Y. 10021.

The opinions or views expressed by contributors do not constitute endorsement or approval by the U.S. Government, DHEW, PHS, Communicable Disease Center.

REPORT OF SUBCOMMITTEE ON EXCHANGE OF INFORMATION ON THE ARTHROPOD-BORNE VIRUSES, FEBRUARY 5, 1964

A total of 88 catalogues have now been issued: 35 to institutions and laboratories within the Continental United States, one in Canada, and 52 overseas, representing a total of 32 countries.

During the last half of 1963, two cards of previously unregistered viruses, namely Stratford and Itaporanga, were issued, bringing the total of viruses now in the catalogue to 141.

Several other registrations are now pending, including the tick-borne encephalitis viruses isolated in the Soviet Union and eastern Europe and vesicular stomatitis, Indiana serotype. Tentative registrations were sent in and have been retyped and returned for approval. Final approval has not been received as yet, so the issuance of cards is being held in abeyance.

During the latter half of 1963, 359 abstracts and current information were issued on 3 x 5" slips. This makes a total of 2,426 issued to date.

As reported in the last issue (No. 8) of the Information Exchange, a questionnaire was circulated to all recipients of the catalogue on the extent of its use and whether or not it should be continued. The responses have been unanimous for the continuance of the catalogue and 90% stated that the associated abstract and current information service was used and all of these favored continuation of it.

One of the reasons for conducting this survey was because it was felt that considerable improvement could be made in the registration form and the key for punching and sorting the cards. Having received this mandate of approval for continuation of the catalogue, the subcommittee has proceeded to revise the registration form and to circulate to all recipients of the catalogue for opinions and suggestions. While, as might be expected, it has not been possible to reach complete agreement on all details, a compromise has been reached which, with few exceptions, has been approved by all consulted.

The next step was to ascertain if those who had registered viruses in the Catalogue, or had agreed to furnish current information on viruses assigned to them, would be willing to cooperate in the re-registration of the respective viruses on the revised registration form. Re-registration is necessary not only because of rearrangement of the questionnaire and

the request for additional information, but also because of the modifications in the key for sorting. So far no one has refused to cooperate and we now have replies covering all but eight of the viruses now registered in the catalogue. The revised forms will be distributed shortly. Anyone wishing to register a "new" virus should apply to the Chairman of the Subcommittee for the revised registration form.

At the last meeting of the ACAV, Dr. W.F. Scherer was elected Chairman to replace Dr. W.C. Reeves, whose term on the committee expired. Dr. Scherer felt that he should no longer continue to serve on the Subcommittee for Information Exchange and he has been replaced by Dr. R.E. Shope at Belem.

REPORT OF SUBCOMMITTEE ON THE RELATION OF BIRDS TO ARTHROPOD-BORNE VIRUSES

Report on meeting at Chicago, Illinois, November 5, 1963. Members present: Drs. Worth, Davis, Hayes, Hickey, Provost, and Stamm.

The manuscript entitled, "Relations of Birds and Arboviruses", by Stamm, Davis, Hickey, Newman, and Provost, was discussed briefly. The few final editorial changes that were suggested have been made by Dr. Stamm, and the manuscript has been submitted to the Auk for publication. It was agreed that the galley proof should be sent to Dr. Newman and that his editing of it will be final.

Dr. Davis' most recent revision of the manual that he is preparing was reviewed in detail. It is entitled, "Manual for Studies for Avian Reservoirs of Arboviruses". Dr. Davis plans to have another draft ready for circulation and review in the spring of 1964.

A number of individuals who might be considered for future membership on the subcommittee were mentioned. It was noted that some effort should be made to obtain an ornithologist who would be representative of workers in the western United States.

The status of the information exchange between ornithologists and arbovirus workers was discussed. It was noted that the International Ornithological Congress symposium will be published next month and that the work of this subcommittee is mentioned in that report. The previously mentioned paper, which is to be published in the Auk, also will contribute information on the subject of birds and arboviruses. In addition, it was suggested that the chairman prepare a listing

of the arbovirus research stations which include studies on birds and a notation of their special project interests. The listing of such stations that are located in the United States is to be submitted for publication to Bird Banding and a more inclusive listing of such stations throughout the world is to be submitted to The Ring.

It was suggested that the interests and purposes of the subcommittee be brought to the attention of the various agricultural experiment station regional offices.

It is planned to request arrangements for showing of the CDC film on arthropod-borne encephalitis and the scheduling of an informal evening discussion session on the relation of birds to arthropod-borne viruses at the next annual AOU meeting. That meeting is to be held in August 1964 at Lawrence, Kansas.

Dr. Davis indicated that he will look into the possibility of having a graduate student initiate a project on a literature survey regarding the criteria for aging birds. He indicated that this should not discourage a similar effort by others, since he does not currently have any particular person in mind for the project.

A note is to be prepared and submitted as a news item to the Wilson Bulletin regarding the changes in the Subcommittee membership.

The next meeting of the American Committee on Arthropod-borne Viruses is to be held in conjunction with the American Society of Tropical Medicine and Hygiene in early November 1964 at New York City. Details of the meeting time and place will be submitted for publication in ornithological publications, especially those in the eastern United States, to advise interested individuals that they are welcome to attend.

The next meeting of the subcommittee is tentatively scheduled to be held in conjunction with the AOU meetings at the University of Kansas, Lawrence, Kansas, in August 1964.

REPORT FROM DR. RAMIRO MARTINEZ SILVA,
COMMUNICABLE DISEASE BRANCH,
PAN AMERICAN HEALTH ORGANIZATION, WASHINGTON, D.C.

During the second meeting of the Advisory Committee on Medical Research of PAHO, which took place in Washington, D.C., 17-21 June, it was suggested that the Director-General should be asked to consider designating the Adolfo Lutz Institute, Sao Paulo, as a WHO Regional Reference Center for Arboviruses.

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

Establishment of a New WHO Regional Reference Laboratory:

The Institute Pasteur, 36 Avenue Pasteur (Boite Postale 220), Dakar, Senegal, has been designated as WHO Regional Arbovirus Reference Laboratory. Dr. P. Bres, Chief of the Arbovirus Laboratory of the Institute, is in charge of the Regional Laboratory.

NOTICE:

The Institute of International Medicine, University of Maryland School of Medicine, announces the availability of postdoctoral research associateships in virology, medical entomology and malariology and parasitology. Appointments can be made at any time of the year. Research associates are appointed for a 3-year period, one year of which is spent at the Institute in Baltimore and two years at the Institute's Pakistan Medical Research Center in Lahore, West Pakistan. Basic salaries range from $5,000 to $10,000 per annum and during the period of residence overseas a 25 per cent overseas differential is added; housing is provided free. An American school covering elementary and high school grades is available for dependents in Lahore. For further information, write: Dr. Herbert C. Barnett, Division of Medical Entomology and Ecology, Institute of International Medicine, University of Maryland School of Medicine, 29 South Greene Street, Baltimore, Maryland 21201.

REPORT FROM THE ARBOVIRUS UNIT OF THE PROVINCIAL LABORATORY, EDMONTON, ALBERTA

Preliminary Arbovirus Studies in Alberta, Canada:

On September 1, 1963, the newly established Arbovirus Unit of the Virology Department of the Provincial Laboratory of Public Health initiated studies on the ecology of arboviruses and on their epidemiological importance to public health in Alberta.

In anticipation of this development, chicken bloods had been collected throughout the summer. Three flocks were established in Lethbridge, a suspected endemic zone in the southeast of the Province. These birds were bled monthly. This was expected to give some indication of the seasonal degree of virus distribution in that area. To obtain preliminary information about other areas of the Province, bloods were collected from chickens coming to a large packing plant in Edmonton. The source and age of these birds were known and a good area distribution was achieved by random selection. From Lethbridge, 410 bloods were obtained, and 340 from other areas. Chicken sera were maintained at -70° C. until tested. After acetone extraction, the sera were tested by the HI method for the presence of WEE, EEE, and SLE antibodies. No definitive evidence of EEE and SLE antibodies was detected. The results of the tests with WEE virus are shown in Table 1.

In August and September a considerable incidence of encephalitic infections in horses and humans was reported from an area of the Province a little south and east of Lethbridge. Specimens were received from 31 horses and 7 humans. Equine blood sera were tested by HI and CF tests for evidence of recent infection (Table 2) and the human specimens by CF test only (Table 3). Blood clots from human cases were inoculated in eggs and mice. Virus was not isolated.

The results of the preliminary survey have led to plans for more detailed investigation. This will consist in the establishment of sentinel chicken flocks in selected locations in the north, northeast, east, south, and west areas of the Province. These birds will all be bled monthly and HI tests will be carried out as before. In addition, arrangements have been made to collect mosquitoes from the south of the Province for virological studies.

TABLE 1.

HI Results with WEE Virus in Chicken Sera

Location	Feb.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Lethbridge	None	*0/53	0/84	0/74	11/130	15/69	None	None	None	26/410
Other areas	0/44	0/42	None	0/31	0/45	0/58	6/83	4/27	2/10	12/340

*No. positive/No. tested

TABLE 2

CF and HI Results with WEE Virus in Horse Sera

Animals Tested	Positive by CF	Positive by HI	Animals with 4-fold rise in CF Antibody
31	24	27	1/3*

* Paired blood specimens received from only 3 horses.

TABLE 3

CF results with WEE virus in 7 patients clinically diagnosed as WE encephalomyelitis.

Patient's Name and Age	Location	Onset of Disease	Date Blood Specimens Received	CF Titer
W. H., 32	Hanna	Aug. 24	Sept. 3	< 8
			Sept. 18	8
			Oct. 5	32
S. J., 20	Cranford	Aug. 24 (?)	Sept. 3	< 8
			Sept. 21	16
			Oct. 17	32
K. R., 17	Coaldale	Sept. 1	Sept. 9	< 8
			Sept. 23	16
			Oct. 19	32
M. M., 1 yr.	Cereal	Sept. 5	Sept. 11	< 8
			Oct. 28	16
P. R., 41	Medicine Hat	Aug. 29	Sept. 12	< 8
			Sept. 24	32
			Oct. 11	32
T. L., 29	Medicine Hat	Sept. 2	Sept. 12	8
			Sept. 24	8
B. W., 41	Medicine Hat	Aug. 28	Sept. 12	< 8
			Sept. 24	< 8

REPORT FROM DR. RUTH MOFFATT, A.D.R.I., ANIMAL PATHOLOGY DIVISION, CANADA DEPARTMENT OF AGRICULTURE, LETHBRIDGE, ALBERTA, CANADA

Large numbers of cases of western equine encephalomyelitis appearing in southern Alberta and British Columbia in 1961, and again in 1963 prompted the initiation of studies on the disease here. During the period from May 1, 1963, to October 30, 1963, 51 suspected cases were reported to the Institute, of which 17 were confirmed. Fourteen of those were confirmed by serological examination of paired blood samples, the remaining three by virus isolation from brain material.

Comparison of the strain of WEE maintained at our main laboratory in Hull, Quebec, showed some variation in characteristics to that isolated here in Lethbridge in 1961. The Lethbridge strain appears to be of low pathogenicity for guinea pigs on primary inoculation. In embryonated chicken eggs, it seems to grow equally well at 37°C incubation for 48 hours or at 40°C for 24 hours. Pathogenicity for mice is also lower than that exhibited by the Hull strain. However, egg passage appears to increase virulence. In guinea pigs, even though clinical symptoms were transitory or absent, titres of up to 1/160 were observed with complement fixation tests.

Work is underway at present on neutralization studies of these two strains using fowl sera prepared against them, and the embryonated egg as a medium. So far we have observed 2-3 logs protection of the sera against homologous virus, but little or none on cross-neutralization tests. The brain material from positive cases in 1963 is being held for future work on identification. Diagnosis was made by egg passage and subsequent inoculation and bleeding of guinea pigs.

REPORT FROM THE DEPARTMENT OF VETERINARY SCIENCE, UNIVERSITY OF SASKATCHEWAN, SASKATOON; PROVINCIAL PUBLIC HEALTH LABORATORY, DEPARTMENT OF PUBLIC HEALTH, REGINA; ENTOMOLOGY RESEARCH INSTITUTE, RESEARCH BRANCH, CANADA AGRICULTURE, OTTAWA

Arbovirus Investigations in Saskatchewan

Study of the arboviruses in Saskatchewan has continued since our last report (Infoexchange #7, March 1963). The summer of 1963 in the Province was abnormally warm and wet with the largest outbreak of mosquitoes in many years and epidemics of WEE in man and horses.

Mosquito Investigations, 1963 - J. McLintock

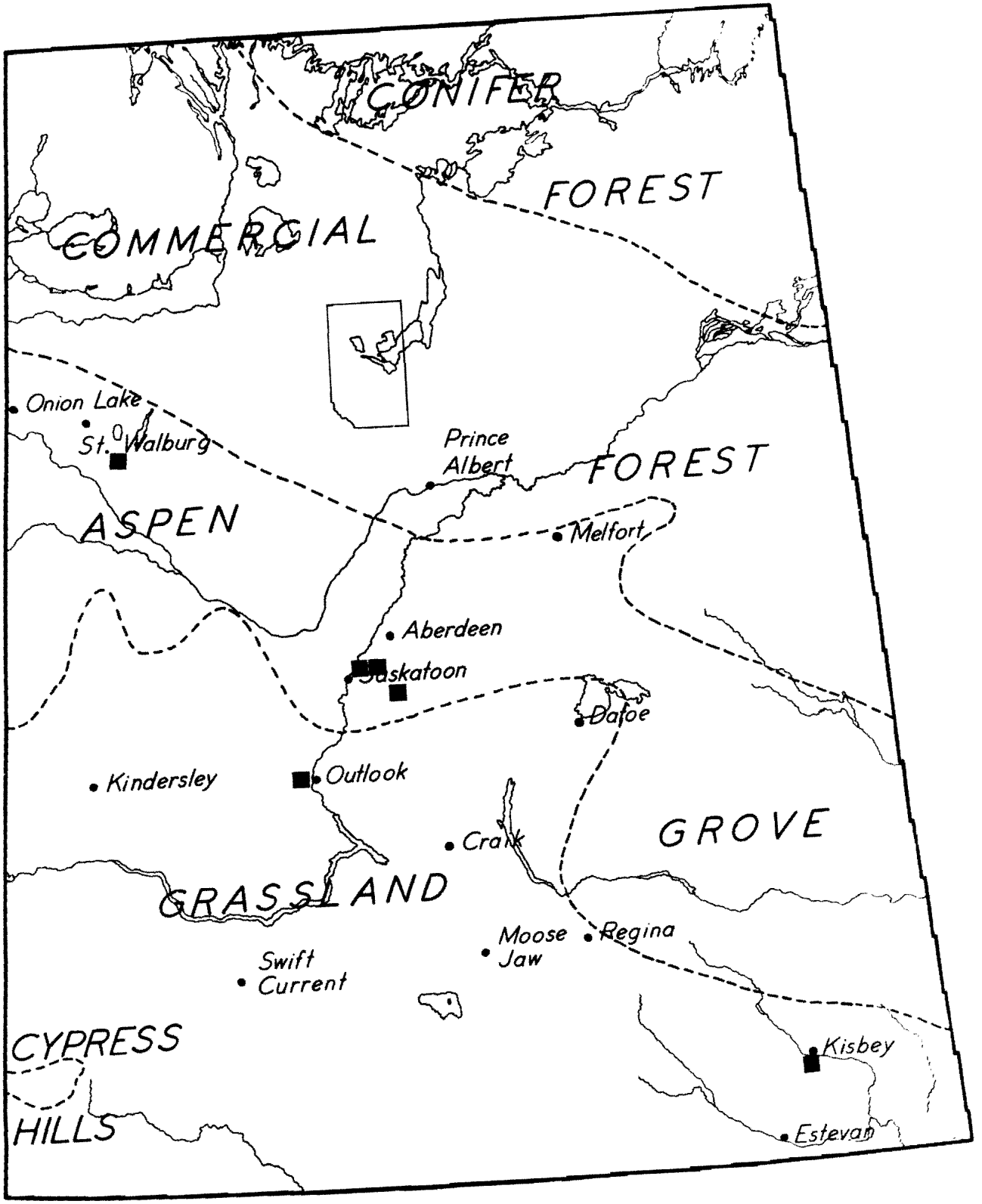
The summer of 1963 was our first complete season of the mosquito survey. Six permanent trapping sites were established (see map, where the black squares represent the trap sites) and these were operated each night from June 1 to September 30.

In Saskatchewan, the winter of 1962-63 was generally mild and dry and by the end of March, agriculturalists were becoming concerned about moisture reserves. However, rains began in April and continued through May and June. During July and August heavy thundershowers were frequent and although a dry period began in September, by the end of that month precipitation from April 1 was still about 300 per cent above normal. At no time during June, July, or August was there any noticeable drying of sloughs.

During April, mean temperatures were two to three degrees above normal but, except for the last week of May, both May and June were generally cool. However, during the first week of July, mean weekly temperatures began to rise, and in four out of our six collecting districts mean weekly temperatures remained above normal for the following 13 weeks, i.e., until the end of September. At the other two stations (Melfort and Swift Current) there were brief drops below normal during two weeks.

On the Praries of Western Canada a wet season is likely to produce an abundance of the univoltine aedines and a hot season an abundance of the multivoltine species. Consequently when the season is both wet and hot, conditions can be favorable for the production of large numbers of all species and this happened during the summer of 1963 in Saskatchewan. The number of trap nights was 2-1/2 times greater in 1963 than in 1962 but the number of female mosquitoes taken was 8 times more than in 1962. In addition, both univoltine and multivoltine species reached their seasonal peaks at about the same time, from the third week of July till the middle of August.

When the survey traps started for the season on June 1, mosquitoes were not noticeably abundant and the univoltine aedines, particularly *Aedes campestris*, predominated in the catches. The multivoltine *A. dorsalis* and overwintered females of *Culiseta inornata* were also present and the first generation of *C. inornata* of the season began to appear during


CONIFER
FOREST
COMMERCIAL
Onion Lake
St. Walburg
Prince Albert
FOREST
ASPEN
Melfort
Aberdeen
Saskatoon
Dafoe
Kindersley
Outlook
GROVE
Craik
GRASSLAND
Moose Jaw
Regina
Swift Current
CYPRESS
HILLS
Kisbey
Estevan

that first week. By the third week of June, univoltine and multivoltine species were appearing in about equal numbers in the traps. The first males of Culex tarsalis (representing the first generation of the current season) appeared in the traps from July 2 to 27, a month later than C. inornata, and the presence of C. tarsalis did not begin to have a noticeable effect on the size of the catches until the latter half of July. By that time the multivoltine species were the most abundant in all the traps, but the univoltine aedines had also been increasing and both groups reached their maxima between July 22 and August 12.

Table 1 gives the relative abundance of the females of the 22 species taken in our survey light traps and in our miscellaneous collections (by aspirator and by light trap + CO_2). From these, 46,283 mosquitoes in 1844 species pools were preserved for examination for virus.

We recently received from Dr. A.E.R. Downe, Department of Entomology, University of Kansas, Manhattan, the results of precipitin tests done on mosquitoes submitted to him from our 1962 collections. These are summarized in Table 2. Of particular interest regarding the epidemiology of WEE was the multiple feedings indicated for C. inornata and C. tarsalis.

Table 1. Relative Abundance of Female Mosquitoes in Southern Saskatchewan, June 1 - Sept. 30, 1963.

Species	Melfort		Saskatoon		Delisle		Outlook		Craik		Swift Current		All Light Traps		Miscellaneous* Catches	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Culiseta inornata	4654	62.2	2147	43.9	19764	46.9	21895	61.4	326	17.7	979	34.2	49765	52.5	4366	27.4
Aedes dorsalis	610	8.1	904	18.5	10832	25.7	8033	22.5	408	22.2	630	22.0	21417	22.6	2880	18.1
Aedes campestris	412	5.5	551	11.3	5142	12.2	2063	5.8	186	10.1	173	6.0	8527	8.9	1046	6.5
Culex tarsalis	712	9.5	549	10.5	1681	3.8	1009	2.8	596	32.4	500	17.5	5047	5.3	2588	16.3
Aedes spencerii	202	2.7	38	<1	1403	3.3	505	1.4	31	1.7	48	1.7	2227	2.3	2133	13.4
Aedes vexans	336	4.5	184	3.8	245	<1	419	1.2	104	5.7	107	3.7	1395	1.5	1242	7.8
Aedes nigromaculis	25	.3	9	<1	129	<1	218	<1	90	4.9	123	4.3	594	<1	185	1.2
Aedes flavescens	205	2.7	33	<1	138	<1	98		23	1.3	23	<1	520		371	2.3
Anopheles earlei	19	.3	58	1.2	24	<1	10		2	<1	3		116		202	1.3
Aedes stimulans	20	.3	12	<1	2		6		7		11		58		8	<1
Aedes fitchii	31	.4	4		7		4		2		4		52		22	<1
Aedes cataphylla	8	<1	3								28		39		29	<1
Aedes punctor	13	<1	6		2				2		2		25		94	<1
Aedes riparius	2		3		1		2						8		31	
Aedes excrucians	2				5								7		14	
Aedes increpitus	4												4		13	
Culiseta minnesotae	3												3		1	
Culiseta morsitans	3												3		1	
Aedes canadensis	1												1		2	
Aedes cinereus	1												1		0	
Culex restuans							1						1		0	
Culex territans			1										1		1	
Mansonia perturbans															2	
Indeterminates	224	3.0	386	7.9	2759	6.5	1393	3.9	61	3.3	229	8.0	5052	5.3	677	4.3
Totals	7487		4888		42134		35655		1839		2860		94863		15908	

*Miscellaneous catches = Catches by aspirator, traps baited with CO_2, and with light + CO_2.

Table 2. Summary of Precipitin Tests on Mosquitoes from Saskatchewan, 1962.

Species	No. Tested	Precipitin-test Result*
C. inornata	40	12 B, 1 Hu, 1 S, 2 Hu F, 2 B F, 1 H D, 1 Hu Un, 1 S Un, 1 B Un, 1 Hu H R F, 1 H P B, 2 Un**, 14 NT
A. nigromaculis	4	1 B, 3 NT
A. vexans	1	1 B
A. campestris	1	1 NT
A. punctor	1	1 F
A. dorsalis	2	1 B, 1 F
Culex tarsalis	8	2 B, 2 F, 1 B R, 1 Hu F, 1 S F, 1 B F

*B = Beef; D = Dog; F = Bird; H = Horse; Hu = Human; P = Pig; R = Rodent; S = Sheep; Un = Unidentified meal; NT = no precipitin test and negative chemical test for blood.

**No precipitin test, but a positive benzidine test for blood.

Antibody Detection and Virus Isolations
(R. Connell, A. Burton, J. Spalatin)

Virus Isolations:

Examination of 738 pools of mosquitoes collected in Saskatchewan during the summer of 1963 yielded three isolations of WEE virus. All isolations were from *Culex tarsalis*. Other species examined are mentioned in Dr. McLintock's section of the report above. A number of mosquito pools from the 1963 collection are yet to be examined.

In 1963, studies of wild birds for the presence of WEE virus, an effort was made to collect as many samples as possible from nesting English sparrows (*Passer domesticus*), since 12 isolations of WEE virus were made from the blood of this species in 1962. A total of 119 blood samples collected in 1963 from nestling sparrows have been examined to date, 8 isolations of WEE virus have been obtained. One isolation of WEE was also made from a domestic fowl in a sentinel chicken flock.

Sentinel Chicken Flocks:

Data concerning sentinel chicken flocks under study in 1963 are not complete. In one flock, 21 of the 24 birds in the flock developed positive antibody titers. It was from a cockerel in this flock that the virus isolation mentioned in the previous paragraph was made.

WEE Neutralizing Antibody:

Neutralizing antibodies were found in blood from a small number of animal species. Species yielding positive results in 1963 are tabulated below. Included in the table are some 1962 results that were not presented previously.

Species	Year	Total Examined	Positive	Suspicious	Negative
Thamnophis spp.	1963	366	28	84	254
Rana pipiens	1962	266	30	53	184
Passer domesticus	1962	45	3	6	36
" "	1963	77	4	16	57
Corvus b. brachyrhynchos	1963	70	3	6	61
Swallows (3 spp.)	1963	51	1	8	42
Pica p. hudsonica	1963	37	2	5	30
Sturnus vulgaris	1963	34	4	5	25
Hawks (various spp.)	1962-63	23	4	1	18

Other species not listed in the table yielded negative results.

All positive frogs were from two small habitats. From the one a total of 20 frogs (14 positive, six negative) were collected August 31, 1962. A few days later (September 4 to 7, 1962), 113 frogs were collected at the second site which was more than 300 miles from the first. Of these, 16 were positive and 33 suspicious. The mode of exposure to WEE virus in these two widely separated frog habitats is not known.

WEE in Man in Saskatchewan, 1963 (H. Dillenberg)

The first human cases were reported on August 2, and the last early in October. Thirty-seven human cases of WEE were confirmed by laboratory tests. Four deaths occurred due to or in connection with WEE, all in elderly men. From one of these, and from the throat washings of an infant, WEE virus was isolated. In 31 cases, the evidence rested upon WEE-positive complement-fixation tests.

More cases of WEE (probably 80-100) were suspected on clinical grounds but laboratory confirmation was either impossible due to unsuitable timing of sample submissions, or were not requested at all. Over 60 cases of encephalitis were reported by hospitals where confirmation was not available. Many of these might have been cases of WEE, as the following complement-fixing antibody survey indicated.

Two districts were chosen for the survey, the Outlook district with a population of 22,600 and the Weyburn district (70 mi. southeast of Regina and 54 mi. northwest of Estevan, see map) with a population of 58,569. WEE is known to be endemic in the Weyburn district. From January to November, 1963, 3001 blood samples were tested and were obtained as follows. From the Red Cross, 484 serum samples were obtained from the Outlook district and 1456 from the Weyburn district; premarital blood specimens provided 329 from the Outlook district and 732 from the Weyburn district. Since the first human cases of WEE were reported on August 2, the study was divided into two parts, the first comprising samples collected up to July 31 (2095) and the second, samples obtained after August 1 (906).

Results

Outlook district -	Before July 31	- 351	persons	tested	- 7 +ve=2.0%
	After Aug. 1	- 462	"	"	- 4 +ve=0.9%
Weyburn district -	Before July 31	- 1744	"	"	- 31 +ve=1.8%
	After Aug. 1	- 444	"	"	- 32 +ve=7.2%

No clinical cases of WEE were reported from the Outlook district in 1963 whereas many clinical cases were reported from around Weyburn. Projected upon the populations of both districts, the above figures indicated 430 inapparent recent infections in the Outlook district and 950 in the Weyburn district before the 1963 outbreak. Following the outbreak, only 180 inapparent infections could be assumed in the Outlook district but over 4000 in the endemic Weyburn district.

REPORT FROM DR. ROBERT P. HANSON
DEPARTMENT OF VETERINARY SCIENCE
UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

Pheasants and rabbits used as sentinels and attempts at isolation of virus from arthropods and vertebrates revealed 1963 to be a quiet year for arthropod-borne viruses in Wisconsin.

Serology in Migratory Birds, Ontario. Approximately 1,000 samples from migratory birds that had been collected at Long Point, Ontario, in the spring or in the autumn of 1962 by Dr. L. Karstad of Ontario Veterinary College, Guelph, were tested for neutralizing antibodies to EEV, WEV, and SLE. The metabolic inhibition test was used. In the spring, 2 serums contained neutralizing substances, a blue jay for St. Louis encephalitis (1 of 17 blue jays tested) and a catbird for eastern encephalitis virus (1 of 13 catbirds tested). In the autumn, 5 serums contained neutralizing substances for St. Louis encephalitis virus. These and the number of each species tested were: Slate-colored Junco 1 of 20, Olive-backed Thrush 1 of 15, Hermit Thrush 1 of 6, Song Sparrow 1 of 4, Rufus-sided Towhee 1 of 2.

Human Serology, Georgia. Serums of 81 adult men employed in outdoor activities during the entire year were obtained from the Georgia Department of Public Health. In addition, serums of 73 candidates for the high school football team, Waycross, Georgia, were drawn at about the same time. These serums were subjected to the metabolic inhibition test in this laboratory. Results based on a single test are as follows:

	Sera of Adult Woodsmen Positive	%	Sera of High School Boys Positive	%
EEE	6/81	7	0/73	0
WEV	7/81	8	1/73	1
SLE	23/81	28	0/73	0
EMC	9/81	11	3/73	4
VSV (NJ)	26/81	32	1/73	1
CEV	42/81	52	11/73	15

Vesicular Stomatitis in Georgia. In three successive years--1960, 1961, and 1962--epizootics of vesicular stomatitis (VS) occurred in northwestern Georgia. Prior to the VSV epizootic season of 1963, 12 dairy herds in northwestern Georgia were selected for vaccination on the basis of their disease history. Approximately 60 per cent of the 556 animals in these herds had VSV-NJ neutralizing antibodies.

States	No. herds	Percent reactors 1963
Disease history 1962	5	86
No history 1962	7	24

A portion of 82 additional herds was vaccinated during the 1963 epizootic of VSV in Georgia. Thirty-eight per cent of the cattle (517/1352) in herds affected with VSV in July and August, 1963, had demonstrable VSV-NJ antibodies when the epizootic began in July and 15 per cent of the cattle (375/2486) in herds that did not become infected had antibodies in July. It would appear that risk of infection is primarily dependent upon ecological factors.

Thirty per cent of the cattle in non-vaccinated herds developed clinical VSV as compared to only six per cent of the cattle vaccinated during the epizootic. Forty per cent of the clinical cases occurred in the non-vaccinated herds during the first week and 80 per cent had occurred by the end of the second week. In vaccinated cattle, 80 per cent of the clinical VSV cases occurred during the first seven days and 99 per cent had occurred by the end of the second week after vaccination.

Indiana VSV Neutralizing Antibodies in Georgia. Individual animals in one herd of Georgia dairy cattle were reported to have had vesicular stomatitis in 1961 and 1962. Forty seven of the 48 animal serums tested were positive for virus neutralizing antibodies to New Jersey type VSV when screened

in a colorimetric neutralization test (CNT). Five of the 48 animal serums were positive for Indiana type VSV neutralizing antibodies in this same test. Selected serums were studied in a quantitative CNT and chicken embryo neutralization test (CENT). CENT titers (LD_{50} log to base 10 virus neutralized) were 1.6, 1.3, 2.2, 1.5, 1.3, 2.4, 1.5, and 1.3. CNT titers (serum dilutions that neutralized 100 TCD_{50} of virus) were 13, 3, 8, 243, 23, 54, 27, and 9. If these titers are non-specific, they are more persistent and much greater than any previously reported.

Relationship of Indiana VSV Strains and Tr 40233. Serum neutralizing indices of cattle and pony serums collected 3 weeks after inoculation with 4 Indiana type isolates of vesicular stomatitis virus were studied. The 4 isolates were: VS-Lab virus isolated 1925 from cattle in Indiana; VS SJNM virus isolated 1956 from cattle in San Juan Indian Reservation, New Mexico; VS-BT 78 virus isolated 1960 from Phlebotomus sp. in Bocus del Toro Province, Republic of Panama; VS-Tr 40233 (Cocal) virus isolated 1961 from Gigantolaetaps mites in Trinidad.

Apart from individual differences in responsiveness of cattle to vesicular stomatitis virus several observations can be made. Cattle and horses receiving Cocal virus developed neutralizing antibody to Cocal virus alone. All titers were expressed as dilution of serum that neutralized 100 to 500 TCD_{50}. Cattle and horses receiving the other Indiana strains, BT-78, Lab and SJNM, usually developed antibodies that neutralized Cocal virus. The neutralizing indices (averages of 6 observations) of the heterologous antisera for Cocal virus, however, were always low (27) when compared to titers for the homologous virus combinations (685) or other heterologous combinations (313).

Experimental Eastern Encephalitis. The resistance of pen reared pheasants to eastern encephalitis increases rapidly with age.

Titrations of eastern encephalitis virus (Georgia horse brain 1962 isolate) in pheasants of various ages (0.1 ml s.q.):

Age at Inoculation	LD_{50} in Pheasants
Wet pheasant chicks	$10^{-9.5}$
24 hours	$10^{-9.4}$
48 hours	$10^{-9.5}$
96 hours	$10^{-7.8}$
6 days	$10^{-8.8}$
14 days	$10^{-6.1}$
4 weeks	$10^{-4.4}$
6 weeks	$10^{-3.4}$

Studies are continuing on transmission of EEV, WEV, and CEV by Haemaphysalis ticks.

Studies of Snowshoe Hare Populations in Alberta. Since the summer of 1961, Thomas Yuill of our group has been conducting virus studies in populations of snowshoe hares in an area north of Edmonton, Alberta, Canada. This study is being carried out in conjunction with a population study of the hares by Professor L.B. Keith of the Wildlife Department, University of Wisconsin.

Isolations have been made from tick pools, clot suspensions, and a rectal swab. Serological surveillance of the hare populations and a few associated species has been carried out.

One of the 1962 clot isolates proved to be Silverwater Virus. We believe this to be the first isolation of this virus from a vertebrate.

Two EEE-like viruses were isolated. One isolate was obtained from a rectal swab of a snowshoe hare trapped in the Rochester, Alberta, area in April, 1962. This isolate gives identical cross neutralization with our stock EEE (a Georgia Culiseta melanura isolate), but titers two logs higher in mice. The Rochester isolate infected experimental snowshoe hares by oral installation of 0.1 ml of stock virus. Hares so infected developed a viremia lasting 24-48 hours.

The second EEE-like isolate was isolated from a clot suspension prepared from a blood sample drawn from a snowshoe hare snared 5 miles west of Notikewin, Alberta. This isolate is neutralized by anti-EEE serum, but the anti-Notikewin isolate serum

neutralizes our Georgia EEE virus poorly (1.2 logs compared to 3.7 logs of neutralization of the homologous Notikewin virus). Serological surveillance indicates that an EEE-like agent was active in the Rochester area in 1961, but not in 1962 or 1963. During these last two years the snowshoe hares suffered a marked population decline.

Neutralization tests indicated that a California encephalitis group virus was active in the area (over 90% of the adult cohort having antibodies in the summer of 1962).

A substantial increase in anti-WEE antibodies occurred in snowshoe hares and Franklin's ground squirrels in May, 1963. An outbreak of WEE occurred to the south of our area in the prairies and transitional parklands of Alberta and Saskatchewan the following July, August, and September.

REPORT FROM DR. EDWIN W. JENNEY, U.S. DEPARTMENT OF AGRICULTURE NATIONAL ANIMAL DISEASE LABORATORY, AMES, IOWA

Vesicular stomatitis (VS) epidemiological studies were resumed in Georgia during the summer of 1963 by Dr. Lloyd D. Colony, a veterinary epidemiologist, and Gerald R. Sutter, an entomologist.

The study consisted of insect trappings from the affected area and an epidemiological survey of infected premises. The tentative five-county study area was enlarged to include an adjacent area experiencing a high incidence of New Jersey type VS. Simultaneous vaccination studies were conducted by the University of Wisconsin. Studies were made in cooperation with the Georgia Department of Agriculture and Georgia Animal Disease Eradication personnel.

In Heard County during 1961 the first infected animal was found three miles from the herd which reported the first infection in 1962 and 1963. In Talbott County, the same farm reported the first VS in 1961 and 1963; this case was also the first reported in Georgia during 1963.

Individual records have been kept in selected herds to determine the age at first infection and the record of re-infection. "Pre and post season" serum samples were collected from selected herds which will be studied for five years.

In two herds, teat lesions were observed and later in the outbreak mouth lesions appeared. The epidemiologist indicated that frequently teat lesions may be followed in one to three weeks by mouth lesions in the same animal. Sixty-five of eighty-eight insect pools collected have been negative on I.C. inoculation into litters of 1-2 day old suckling mice. Insect pools consisted mostly of mosquitoes with some pools of Siphona, Tabanus, Chrysops, Musca, Simulidae, and ticks. No Phlebotomus were detected during the study. Processing the insect pools was delayed during the summer by diagnostic activities and interrupted for alterations of animal facilities, but is expected to be resumed by mid-February.

VS studies in Georgia are a part-time project by Diagnostic Services of the Animal Disease Eradication Division. Insects and serums collected during the summer are processed at NADL during the fall and winter following the diagnostic load of the VS season.

In 1963 NJ type VS was first reported in West Central Georgia in mid June and reached a peak in July and August. Although spreading in all directions, the VS outbreak extended westerly two thirds of the way across Central Alabama before terminating in December. Georgia had 292 laboratory confirmed infected herds, Alabama 64, South Carolina 5, Arkansas 4, and Florida 1.

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

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

Epidemiologic Investigation of 1963 Texas Encephalitis Outbreak

Further information is now available on the outbreak of acute febrile central nervous system disease which occurred in the High Plains area of Texas in the summer of 1963. The outbreak began in the human population in the last week in June and reached a peak during the week of 8-12 August. The onset of the last reported case was 30 September. Eighty-seven human cases were similar on clinical grounds, yet only 27 of 70 patients studied were considered confirmed or presumptive WE cases. Recent infection with WE or SLE could not be confirmed in any of the remaining patients from whom serum

specimens were obtained. It seems probable that this encephalitis outbreak was caused by WE virus and another as yet undefined etiologic agent. Serologic tests have indicated Coxsackie virus activity in certain of these patients, but these studies are still in progress.

Virus Isolations from Texas Mosquitoes, 1963

During the recent encephalitis outbreak in Texas, WE virus was isolated from 58 per cent of the mosquito pools collected on 19-22 August, and from 29 per cent of those collected on 23-27 August. By the first week in September, the percentage of pools positive for WE had dropped to 13 per cent; less than one per cent contained WE virus during the 13 September-7 October period. SLE virus was isolated at least twice and four other isolates may prove to be SLE. Five isolates appear to be related to an unidentified arbovirus first isolated in 1961 from six pools of Texas mosquitoes, and subsequently isolated from four more pools in 1962. Twenty-two isolates appear to be arboviruses unrelated to any of the other viruses isolated during this outbreak, but appear to be related to each other. In view of the possibility that more than one encephalogenic virus was active in the outbreak, these mosquito isolates are being tested against acute and convalescent human sera.

WE Virus Activity in the Texas Outbreak Area, 1963

Sentinel chickens maintained in Abernathy and in Hale Center, Texas, during the 1963 outbreak were found to have HAI antibody to WE virus in from 75 to 97 per cent of the chickens in five untreated flocks (Table 1). All but one flock of one-year-old chickens from three Abernathy farms and three Plainview farms had comparably high HAI antibody rates to WE, ranging from 64 to 100 per cent; in one flock from Plainview only 12 per cent of the chickens had WE antibody. The frequency of WE-positive chickens is not significantly higher than for the past several years in the same area, even though an outbreak with more than 70 human cases and five deaths occurred. These data, together with mosquito isolations, suggest that WE virus has been quite active every year since 1959 in the Panhandle area of Texas, but some significant change in the arbovirus ecology caused virus to spill over into the human population during 1963.

Prevention of Encephalitis Virus Transmission

During 1963, an attempt was made to reduce encephalitis virus transmission in the Texas Panhandle study area through the use

of outdoor residual sprays of DDT. Four farmsteads (Table 1) were treated with 5 per cent formulations of DDT during May, July, and August, and untreated farmsteads were used for comparison. Results indicate a marked reduction in WE virus transmission in the treated flocks. The percentage of chickens with WE antibody varied from 19 to 35 per cent, whereas the untreated flocks varied from 75 to 97 per cent. These results from Texas, together with the results of the 1962 studies in Washington, provide the first positive indication that mosquito control may be effective in preventing transmission of the arboviruses.

Ecology of Arboviruses in Colorado, 1963

WE and SLE viruses were active again in 1963 in the St. Vrain study area Greeley. Twenty-six and 10 per cent of the chickens had HAI antibody to WE and SLE respectively.

A group of 33 hamsters were held in the St. Vrain site, and 9 per cent developed neutralizing antibody to WE by October. Sixteen rabbits were similarly exposed to natural infections, and two showed an equivocal reaction to WE in the neutralization test. Further serologic study is required to determine whether the mammal sentinels experienced infections with arboviruses not experienced by the chicken flock.

Forty-nine small mammals representing six species were collected in October from the study site, and tested for neutralizing antibody. Of 26 *Peromyscus*, one had definite neutralizing antibody, and another had an equivocal reaction. One of six harvest mice had an equivocal reaction in the neutralization test. Thirty per cent of the magpies collected in the same area had neutralizing antibody to WE virus.

Colorado Tick Fever Studies

Thirty-seven cases of Colorado tick fever were reported in the first seven months of 1963 in Boulder and Larimer Counties, Colorado. An ecologic study was made of all of these cases which could be contacted (62 per cent). Most of the uncontacted persons were tourists or transients who had left the region. Eighty-seven per cent of the clinical histories of the 23 patients who could be contacted were judged clinically consistent with the diagnosis. More males than females were reported (61 per cent male, 39 per cent female). Cases were evenly distributed through all age groups. More than half of the persons reported that they picked up their tick in the estimated 7-8000 ft. elevation region. Two-thirds were engaged

Table 1

Hemagglutination-Inhibiting Antibody in Texas Sentinel Chickens, 1963 Season

Area	HAI Antibody Against					
	WEE McMillan			SLE Parton		
	No. Test.	No. Pos.*	% Pos.	No. Test.	No. Pos.*	% Pos.
Hale Center						
Townsend**	26	9	35	26	0	0
Law	29	23	79	29	2	7
Holland	28	21	75	28	0	0
Engles	28	24	86	28	1	4
Hahn**	26	9	35	28	0	0
Abernathy						
Howard**	26	5	19	26	0	0
Johnson	29	28	97	29	3	1
Neis**	23	7	30	23	1	4
Brown	28	25	89	27	3	11

* Inhibition at dilution of 1:40 or greater
** Treated with DDT in May, July, and August

in recreational activity at the time of contacting the tick, and one-third were engaged in work activities. Over half of the total cases picked up their ticks in the sparse conifer (mostly Ponderosa pine) and heavy underbrush vegetational type, and about one-fourth in the cottonwood and heavy brush stream bottom areas. A striking geographical "clustering" of cases was found, especially in Larimer County.

Study of Non-Specific Hemagglutination Inhibitors in Chicken Serum

For some time, the Disease Ecology Section, Technology Branch, CDC, has been concerned with a peculiarly selective non-specific inhibitor of hemagglutinins (HA) prepared from suckling mouse brains infected with certain strains of WEE virus. Thus far, the inhibitory substance has been detected only in sera of certain adult, female chickens; therefore, for brevity, it will be referred to as CIS.

CIS has the following unusual characteristics: (1) It is not removed by standard methods of acetone extraction, nor is it soluble in diethyl-ether at 4°C; (2) It can be removed by CO_2 precipitation, filtration through Seitz SK filter pads or ultra-fine fritted glass, or by treatment with Kaolin; (3) Aliquots of CIS-positive chicken sera may inhibit Olitsky (WEE) mouse brain antigens in titrations of 1:2560 or greater, yet have no effect on the HA prepared from the same strain of Olitsky virus after 9 passages in half-day-old chicks and extracted from chick plasma pools containing the virus. Infrequently, CIS inhibits HA prepared from mouse brain infected with the McMillan strain of WEE virus; however, titers rarely exceed 1:160. None of the CIS-positive sera has been shown to produce non-specific inhibition of the Parton strain of SLE virus. We have received informal reports from other laboratories that their EEE hemagglutinin was inhibited by CIS or a similar substance in chicken sera.

Evidence that the Olitsky mouse brain HA is not inherently "weak" was found in concurrent tests with the McMillan HA against approximately 100 human sera. No failure of acetone extraction to remove non-specific inhibitors against either antigen was found. In testing more than forty human sera containing neutralizing antibody against WEE virus, the HI titers against McMillan mouse brain antigen were almost invariably two- to fourfold greater than against Olitsky mouse brain antigen.

The non-specific CIS was initially detected in female sentinel chickens used as indicators of arbovirus transmission in nature. At approximately eight weeks of age, these flocks were placed in selected study sites in late May or early June and then bled at monthly intervals from July through October. None of the sera from these chickens inhibited Olitsky HA until late August or September, the months during which chickens usually acquire WEE antibody in the midwest. Once the inhibitor was detected in a chicken, it was usually demonstrable in all subsequent bleedings. Positive rates were occasionally as high as 90 per cent. Based on the HI test alone, the serologic results of testing these sentinel flocks seemed to indicate high rates of WEE virus transmission to chickens in the study area. However, in certain flocks, the HI test results could not be confirmed with virus neutralization tests. Thus, the WEE HI prevalence rate in each flock represented the sum of the CIS-positive sera plus the true WEE antibody rate. Many chickens undoubtedly had both substances in their sera. Since the two could not be differentiated by the HI test alone, the problem assumed major importance in our investigative program.

Based on the sentinel flock studies, it was postulated that the inhibitory substance developed in the serum of certain chickens coincidentally with sexual maturity. This hypothesis has been confirmed. Furthermore, the substance has been found only in adult, female chickens; never in roosters. Limited observations suggest that the prevalence of CIS varies directly with the egg-laying rate in chicken flocks.

The chemical nature of the inhibitor has not been determined. However, studies are in progress to accomplish this objective.

This problem is described in the Information Exchange to serve as a precaution to investigators planning to use adult female chickens as indicators of WEE virus transmission rates during the coming season. If antibody rates are determined by the HI test, care must be taken that the antigen employed is not affected by the inhibitor described above. To avoid the problem, it appears that one may use roosters as sentinels, Seitz-filter the serum, or possibly use HA prepared from the plasma of infected 0.5-day-old chicks.

REPORT FROM CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH
DR. EDWIN H. LENNETTE,
CHIEF, VIRAL AND RICKETTSIAL DISEASE LABORATORY, AND
DR. HARALD N. JOHNSON,
DIRECTOR, ROCKEFELLER FOUNDATION ARBOVIRUS STUDY UNIT,
BERKELEY, CALIFORNIA

There were 9 confirmed and 3 presumptive cases of SL encephalitis in California in 1963. There were 2 cases in August, 8 in September, and 2 in October. The earliest onset date was August 7th and the latest October 18. One horse which had encephalitis showed a diagnostic rise of CF antibodies for SL virus and it is presumed that this horse had SL encephalitis. This horse was from Sacramento County and there were 2 cases of SL encephalitis in man in this county at about the same time.

There were 3 cases of WE encephalitis. One was a case of encephalitis in an 8 day old child from Kern County with onset on July 24th. The diagnosis of WE virus infection in the mother is based on a high CF titer when bled a month to 6 weeks after the child developed encephalitis and the fact that the acute blood specimen of the child was negative for CF antibodies for WE virus. The other case of WE encephalitis was from Sacramento County with onset August 21st. There were 6 confirmed and 3 presumptive cases of WE encephalitis in horses. Five of these were from Imperial County with onset between July 15th and August 5th. There were 2 cases in Fresno County which also had 4 cases of SL encephalitis in man.

There were 4 cases of Colorado tick fever in 1963. CTF virus was isolated from the blood in each instance. These patients were exposed to tick bite in the mountains and there was one case each month in May, June, July, and August. CTF virus was isolated from 9 pools of ticks collected May 6-10. Four strains of CTF virus were isolated from ticks collected in Siskiyou County, 4 from Lassen County, and 1 from Modoc County. Eight of the CTF virus isolations were from *Dermacentor andersoni* and 1 from *Dermacentor occidentalis*.

A die-off of *Microtus montanus* was investigated at Tule Lake Wildlife Refuge in Northern California in February 1963. Tularemia organisms were isolated from the lung tissue of 2 mice found dead and from the blood of one *Microtus montanus* trapped at the refuge.

The study of Microtus montanus, meadow mice, at a ranch near Klamath Falls, Oregon, yielded 7 isolations of Rickettsia. All isolations were from blood specimens, 4 from mice trapped in June and 3 from mice trapped in July.

A Tadarida brasiliensis mexicana bat found on the ground unable to fly on October 30, 1963, at Fairfield, Solano County, was sent in for rabies examination to the California State Health Department diagnostic laboratory. It was negative for rabies but the salivary glands were positive for Rio Bravo virus. The brain was negative for virus. A mourning dove found sick in Oakland on September 7th was positive for ornithosis virus. The virus isolation was from brain tissue.

REPORT FROM DR. L. VERNON SCOTT, DEPARTMENT OF MICROBIOLOGY UNIVERSITY OF OKLAHOMA MEDICAL CENTER OKLAHOMA CITY, OKLAHOMA

Studies to determine the role of long range migratory birds in the dissemination of arboviruses have been undertaken at Cheyenne Bottoms, Kansas Wildlife Refuge, Great Bend, Kansas. This is part of a bigger research project which also involves Dr. J. Teague Self, Parasitologist, University of Oklahoma, Norman, who is studying the blood and intestinal parasites of these birds, and Dr. David Parmelee, Kansas State Teacher's College, Emporia, Kansas, who is the ornithologist and interested in many aspects of birds and their habits. Blood specimens collected from long range migratory birds and mammals are being examined for viral content and presence of viral antibodies. Mosquitoes collected in this area are being checked for presence of virus. The serum from sentinel chickens housed in the study area is being checked for viral antibody content. It is hoped that definitive data will be available for the next issue of the Information Exchange.

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

A virus, tentatively identified by the WHO Regional Arbovirus Laboratory at CDC in Atlanta as a strain of Hart Park virus, was isolated from blood of a red-winged blackbird (Agelaius phoeniceus). This was a fledgling taken July 19, 1963, from a nest in shrub at Lake Mermet in southern Illinois.

Since the last week of October, 1963, mosquitoes have been collected from resting places in wooden shelters, along overhanging bluffs, in caves and in large holes drilled horizontally in the course of strip coal-mining operations. The following mosquitoes have been collected and processed, and suspensions have been inoculated intracerebrally into newborn mice.

Mosquito	Number
Culex erraticus	5,097
*Culex (Culex) spp	4,019
Anopheles quadrimaculatus	13
Anopheles punctipennis	304
	9,433

*Field collection of females of this subgenus from southern Illinois may not be reliably determined to species.

No virus has been isolated to date (January 29, 1964). Collection of hibernating mosquitoes will continue throughout the winter months.

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

Ecology of Avian Reservoirs:

The regular netting program was continued during the period August 1 through December 20. In addition to netting birds according to the grid in our forest area (132 acres), other habitats in the area, including fields and a swamp, were sampled. A total of 439 birds belonging to 55 species was captured, from which about 175 satisfactory blood sera and 100 smears were obtained.

The first draft of the revision of our "Manual for Analysis of Birds as Reservoirs" has been completed. The final form of this revision is expected to be finished in the near future.

It was found that *Aedes triseriatus* persisted in the netting area as late as October 16, whereas the other species previously recorded were not observed during this period.

REPORT FROM DR. RICHARD O. HAYES
TAUNTON FIELD STATION, COMMUNICABLE DISEASE CENTER
TAUNTON, MASSACHUSETTS

Virus isolation tests were completed on 972 wild bird blood specimens collected between June 4 and October 17, 1963 at our principal study sites. WE virus was isolated in specimens obtained from a catbird, a flicker, and a purple finch. The 3 positive specimens were from birds collected at the dike in Pine Swamp on September 20, 1963.

Studies on wild bird populations were continued. The population indices obtained during 1963 for the breeding population of summer resident species (e.g. black-capped chickadees, northern waterthrushes, and swamp sparrows) was essentially the same as in the previous year. However, there were noticeable declines in the population indices obtained later in the summer and during early autumn among the species that usually congregate within the swamp at that time of year (e.g. Baltimore orioles, catbirds, and robins). At the study site 2 miles from Pine Swamp, the decline in the wild bird population indices tended to be more generalized.

Blood samples taken 3 times per week during July and August from the sentinel chickens in the flock located within Pine Swamp were tested for virus. None of the 843 blood specimens obtained was positive for virus.

Virus tests were completed on 194 pools of the arthropods collected during 1962. Three isolations of WE virus were reported from these tests conducted by the Disease Ecology Section virus laboratory at Greeley, Colorado (partially reported in the previous Infoexchange, page 44). One of 62 pools of *Culex pipiens* and 2 of 34 pools of *Culiseta melanura* were positive for the WE virus. An additional WE isolation from a pool of *C. melanura* also was reported from the arthropods collected during 1962 that are currently being tested at the Massachusetts Department of Public Health virus laboratory.

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

Survey for Arboviruses in South Carolina (Drs. Henderson, Wallis, Karabatsos, and associates):

As a result of preliminary sampling of mosquitoes and birds in Beaufort County, South Carolina, in 1961 and 1962, it was apparent that the area warranted more intensive study for arbovirus activity. Therefore, in the mid-summer period of 1963, field studies were conducted throughout a four-week period that was planned to coincide with the expected peak of virus activity in the mosquito and bird populations (7/20 to 8/19/63).

During this time, portable mosquito light traps were operated at 18 collection stations in Beaufort County. In addition, with the cooperation of U.S. Marine Corps personnel, mosquitoes were caught at routine trapping stations at the Marine Corps Air Station (MCAS) and at the Parris Island Marine Base (PIMB). From MCAS, 589 mosquitoes (11 species) were obtained, identified and sorted into 49 pools. From PIMB, 1,398 (9 species) were obtained and sorted into 65 pools. While a portion of the Beaufort County material is stored frozen awaiting identification, sorting, and pooling, 24,271 specimens (22 species) have been processed into 720 pools to date. Virus isolation studies have included inoculation of these pools into infant mice and into primary cultures of chick embryo. Arbovirus isolations made thus far from part of the mosquito collection of 1963 are shown in the following table:

Arbovirus Isolations from Mosquitoes Collected in Beaufort County, S.C., 1963

Mosquito Species	Number in Pool	Date Collected	Where Collected	Identity by Plaque Reduction Neutralization
Aedes taeniorhynchus	100	7-27	MCAS	WEE
Psorophora confinnis	63	7-29	MCAS	WEE
Anopheles crucians	1	8-1	MCAS	WEE
Psorophora ciliata	1	8-8	PIMB	WEE
Psorophora confinnis	7	8-10	PIMB	WEE
Aedes taeniorhynchus	107	7-30	Ladies Island	SLE*
Aedes taeniorhynchus	57	7-30	Hunting Island	WEE

*Tentative

Aruac, Iere, and Triniti Viruses (Dr. Downs):

Hyperimmune mouse ascitic fluids were prepared using Aruac (TRVL 9223), Iere (TRVL 8762), and Triniti (TRVL 7994) viruses. These viruses have been tentatively referred to in unofficial publications as constituting a "Triniti group", this classification being based on CF tests. The materials referred to above were cross-checked with antigens (both crude virus suspensions in borate saline buffer and sucrose acetone extracted antigens) in CF. High titered homologous systems were demonstrated with Aruac and Triniti and lower titered for Iere. There was no evidence of a heterologous cross. This study provided no evidence to support the "Triniti group" concept, and it is considered advisable to regard Aruac, Iere, and Triniti as unrelated agents. Indeed each one remains with no demonstrated relationship to any other recognized virus.

Tacaribe Virus Antibodies (Dr. Downs)

Tacaribe virus neutralization tests have been carried out in the Rhesus monkey kidney cell system against 46 human serum specimens from residents of Port-of-Spain, Trinidad. No positives were encountered. Tests have also been completed against 40 Trinidad bat sera, from several different bat species. Three reactions which might be considered as reactions of partial protection (0.9 log reduction in plaque inhibition test) are difficult to interpret because of serum dilution factors.

Treatment of Sera with CO_2 for Removing Non-specific Inhibitors (Dr. Downs):

With the technical assistance of Mrs. Gerda Roze, a study was made of the effectiveness of CO_2 treatment of serum for removal of non-specific inhibitors from sera being tested in HI reaction. The simplicity of the technic is appealing, as contrasted with a laborious procedure such as acetone extraction of serum. Briefly, a 1:10 serum dilution in distilled water in a test tube is overlaid with CO_2 gas for a minute or more. The resulting precipitate is removed by centrifugation and the supernatant fluid tested in HI. Various sera were tested including mouse hyperimmune sera versus various viruses, supplied by Dr. Jordi Casals, and rabbit, goat, chicken, rat, and ferret WEE immune sera supplied by Dr. Albo Saturno. With the WEE immune sera the CO_2 extraction gave one tube lower titer with WEE antigen

than did the acetone extracted sera. The CO_2 extracted sera (even extracted normal sera of several species) retained Group B inhibitors, presumably non-specific. The technic cannot be recommended for routine use in arbovirus work.

Biologic Variation of a Mayaro Virus Strain (Dr. Karabatsos):

Large (>3.0 mm diameter) and small (1.0 mm or less in diameter) plaque types were noted when a 15th mouse brain passage of Mayaro virus strain Tr 4675, was plated on chick embryo cultures. Both types were plaque purified, and mouse brain stocks were prepared for each. Preliminary results indicate no antigenic differences between the two types upon analysis by cross-neutralization tests. The L plaque type did not produce CPE in chick embryo tube cultures; whereas, the S type induced CPE in 2-3 days. Both plaque types produced CPE in duck embryo tube cultures 2 days after inoculation. Mayaro S appeared to be less pathogenic for infant mice. This plaque type titered 100 LD_{50} lower than L.

Serial passage of both plaque types in various hosts indicated Mayaro S plaque type bred true when passaged in both chick and duck embryo cultures. Passage of L in these two culture systems resulted in the gradual replacement of L by intermediate-sized plaques (<3.0 mm and >1.0 mm) and a fraction of small plaques. Passage of L in infant mice resulted in L plaques but accompanied by a small number of intermediate plaques. Passage of S in mice gave rise to L plaques, and subsequently to intermediate-sized plaques. The proportions of the three plaque types varied greatly from passage to passage in mice.

Further Studies on Arbovirus Hemolysins (Dr. Karabatsos):

Since the initial observation that EEE and WEE possessed hemolytic activity for 1-day chick red cells, it was of interest to determine if this is a common property of other Group A viruses and of other arboviruses in general. An additional 23 arboviruses from 5 sero-groups (5 Group A, 12 Group B, 3 Group C, 2 Bunyamwera group, 1 California group) were examined for hemolytic activity. Assay of HA antigen preparations of these viruses revealed that all the A, C, Bunyamwera, and California group viruses tested possessed a hemolysin.

Only 5 of the Group B viruses tested showed hemolytic activity. Only Japanese B of these positive Group B viruses showed hemolytic activity initially. It was necessary to introduce modifications of and/or supplements to the basic assay

procedure in order to demonstrate hemolytic activity by those Group B viruses which were eventually shown to be positive. Thus, it has been demonstrated that at least a single member of five different serogroups possess a hemolysin, and it is probable that this may be a common biological property of arboviruses in general.

REPORT FROM DR. JORDI CASALS
ROCKEFELLER FOUNDATION VIRUS LABORATORIES, NEW YORK, N.Y.

Strains of Eastern Equine Encephalitis Virus Isolated Outside America

Isolations of EEE virus outside America have been mentioned or reported as follows: Philippines, Mace, Ott and Cortez, Bull. U.S. Army Medical Dept. 91: 504, 1949; Czechoslovakia, Libikova, Acta Virologica 1: 93, 1957; Thailand, Rudnick and Hammon, SEATO Medical Research Monograph number 2: 24, 1961; Poland, Wrowlewska, personal communication to the author, 1963; and USSR, Ananyan, Symposium on tick-borne encephalitis and other arbovirus infections, abstracts of papers, Moscow and Minsk, page 73, 1963.

In previous studies in this laboratory, it has been shown that the American strains can be divided into two classes by means of a special type of HI test; these classes are designated as North American and South American types. Attempts have now been made to determine the position of the non-American strains with respect to the American ones.

The following strains were available: Thailand, T 172; Poland, Wit; and USSR, K-10. These were generously supplied, respectively, by Drs. Hammon, Wrowlewska, and Ananyan.

The method of study was by means of the HI test, using: simultaneous dilutions of sera and antigens near the end-points; one- or two-injections immune sera, obtained five or six days after immunization; and examination of the rate of reaction between antigen and serum by making determinations at 30 minutes, two hours, and 18 hours after initially mixing the reagents. In the accompanying tables are given illustrative examples of the results obtained. In order to simplify the presentation, only the determination after the antigen-serum mixture was held for two hours at 22° C. is shown.

Table 1 shows the results of the comparison between the Thailand strain, T 172, a North American type strain, NJ 1945 (New Jersey 1945) and a South American type strain, Ar B (Argentinian B). Table 2 has a similar comparison between the Polish strain, Wit, a North American, Alabama and a South American, Ar LL (Argentinian LL) type strains. And in Table 3, the comparison is between Russian K-10 strain, and NJ 1959 (New Jersey 1959) and Ar B strains .

There is no doubt that within the limits of the test, strains T 172, Wit and K-10, belong with the North American type of strains, from which they are indistinguishable.

There has been some question in the past concerning the validity of some of the isolations of EEE virus outside America. The results reported here can be interpreted in one of three ways:

a) The non-American strains are, in fact, serologically identical to the North American type;

b) The non-American strains differ from the North American type but the method used is not sensitive enough to tell them apart;

c) The non-American strains may represent laboratory contaminations.

The presence of antibodies in man, horses and mules, and birds, against EEE in the countries of origin of these strains would contribute greatly to set aside the third interpretation just given; such antibodies as have been reported to exist, need confirmation for specificity. Reports of human or equine outbreaks, traceable to EEE virus, in the corresponding areas are also missing.

Thailand, T 172 strain

Table 1

Serum, in dilutions 1:		Antigen		
		T 172	NJ 1945	Ar B
T 172	1280	64	64	16
	2560	32	16	0
	5120	8	0	0
NJ 1945	2560	64	32	16
	5120	32	32	0
	10240	8	8	0
Ar B	640	16	8	64
	1280	0	0	16
	2560	0	0	8

0= less than 8 units inhibited.

Hemagglutination-Inhibition Test. Number of units of antigen inhibited by the serum in dilutions. The mixtures of serum and antigen were incubated at 22° C. for two hours, before adding the suspensions of red cells.

Poland, Wit strain

Table 2

Serum, in dilutions 1:		Antigen		
		Wit	Alabama	Ar LL
Wit	1280	128	128	16
	2560	64	32	0
	5120	32	8	0
	10240	0	0	0
Alabama	1280	64	64	0
	2560	32	32	0
	5120	16	8	0
	10240	0	0	0
Ar LL	1280	0	0	32
	2560	0	0	8
	5120	0	0	0

0= less than 8 units inhibited.
Explanation as in Table 1.

USSR, K-10 strain

Table 3

Serum, in dilutions 1:		Antigen		
		K-10	NJ 1959	Ar B
K-10	1280	64	32	0
	2560	8	8	0
	5120	0	0	0
NJ 1959	1280	32	32	0
	2560	16	8	0
	5120	0	0	0
Ar B	1280	0*	0*	64
	2560	0	0	16
	5120	0	0	0

0= less than 8 units inhibited.
0*= less than 16 units inhibited.
Rest of explanation, as in Table 1.

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

Inhibition of Viruses by Heparin:

Nahmias and Kibrick (Bacteriological Proceedings, 145, 1963) reported heparin to be inhibitory for Herpes simplex virus. It became of immediate practical concern to learn whether heparin used in our field collection of bird bloods inhibited Eastern Encephalitis (EE) and Western Encephalitis (WE) in viremic birds and whether the amounts used could inhibit virus in the neutralization test and thus be mistaken for neutralizing antibody. The maximum amount present in a neutralization test of a blood collected with equal volumes of 10 mg.% heparin saline when diluted 1:5 in a neutralization test is 2 mg.% or 20γ per ml.

Five times and 12.5 times this amount were used in tests designed to simulate the worst possible conditions of sample storage, conditions which do not actually occur. Mixtures of virus and heparin were incubated at 37° C for various times, frozen and thawed at -20° (as in field sample storage) and frozen at -65°C.

Equal volumes of 10 mg.% heparin did not inhibit EE or WE nor enhance heat inactivation as compared with controls when exposed to 37° for six hours, or when subjected to two cycles of freezing and thawing at -20°C.

Equal volumes of 25 mg.% heparin did inactivate EE by 70% or more but only after prolonged exposure of six hours to a temperature of 37°C. WE was inactivated by 33%. (Plaque Reduction)

It was concluded that under conditions used in field collections and storage of bird sera and the much smaller amounts of heparin present in a neutralization test that significant inactivation of virus by heparin was unlikely, and that it would not be necessary to change our techniques.

Primary Tissue Cultures:

We have had a number of difficult episodes with primary chick embryo tissue cultures this year which have delayed our work on field specimens (and been generally frustrating).

One problem failure of the cells to attach, coincident with a drop in pH, was associated with the first few tubes planted. The cause was finally ascribed to anoxia of the cells while contained in the tubing of the planting device (Cornwall pipette). The volume of the unsuccessful planting coincided with the capacity of the tubing. (It was overcome by continuous mixing of the suspension.) Anoxia and/or accumulation of acids is a probable cause of losses of the whole day's planting (300 plates, 200 tubes) perhaps because in handling much larger volumes, aeration is neglected and acids accumulate. This has apparently been corrected by adding glucose to the Puck's or Dulbecco's salt solutions used during trypsinization and washing, and adjusting the initial pH to 7.6 with NaOH. The object was to enhance cell respiration during the hours required to prepare large numbers of cultures.

In this issue, the report from the Taunton Field Station presents some of the data obtained by this laboratory in a cooperative study.

REPORT FROM DRS. R. WALTER SCHLESINGER AND THOMAS M. STEVENS, DEPARTMENT OF MICROBIOLOGY, RUTGERS MEDICAL SCHOOL NEW BRUNSWICK, NEW JERSEY

Previous reports dealt with (a) the inhibitory effect of agar extract on multiplication of dengue-2 virus, (b) plaque formation by dengue and other group B arboviruses on KB cell monolayers overlaid with methylcellulose, (c) characterization of the agar inhibitor (AI) as a sulfated polysaccharide, (d) partial purification of dengue-2 virus and identification, by cesium chloride density gradient centrifugation, of two distinct HA fractions of which the heavier one (mean density, 1.24) contains 90 to 99 per cent of the plaque-forming units (PFU).

Since the last report, the following studies have been done:

(1) Agar Inhibitor: Partially purified AI has been tested for activity against various group B arboviruses. Results summarized in Table I indicate marked differences among these viruses in sensitivity to AI. In general, sensitivity is associated with unsatisfactory plaque formation on KB cells under agar overlays, while MVE, SLE, and JBE viruses, which are entirely insensitive to AI, form plaques with equal efficiency under agar or methylcellulose. It is likely that greater or lesser sensitivity to AI may ultimately prove to have great value as a genetic marker just as it has in the case of certain enteroviruses.

It has been demonstrated unequivocally that the AI acts by physical combination with virus particles. The complexes, containing virus and inhibitor at a ratio of approximately 2/1 (in kinetic, not molecular, terms), are reversible. These studies are presented in detail in a paper now in press. (See below.)

(2) Characterization of the Infectious and Non-infectious HA Fractions: In an attempt to determine whether the low infectivity of the "light" HA fraction is associated with a reduced RNA content, virus grown in KB cells in the presence of $P^{32}O_4$ was subjected to partial purification. Three fractions were obtained by CsCl density gradient centrifugation. These fractions, termed POOLS I, II, III, represented high, intermediate, and low densities and differed in PFU titer as indicated in Table 2. (It should be noted here that CsCl reduces the total PFU by 95 to 99 per cent of the original.) All three pools were adsorbed on goose RBC which were washed until no further significant label came off. Control experiments showed that the addition of specific antiviral immune serum to the virus-RBC mixture prevented the attachment of any significant amount of isotope to the RBC. In absence of immune serum, the P^{32} attachment to RBC was extraordinarily stable. It was concluded that the material sticking to the RBC was in all likelihood virus. The RBC served as a very efficient selective "receptor" since by far the greater part of the original radioactivity, even after partial purification procedures was obviously non-viral.

Instead of attempting to separate the virus from the RBC, the thoroughly washed virus-RBC complexes were subjected to fractionation as illustrated in Table II. It can be seen that there were marked differences in the distribution of label in the three pools and that the highest proportion in Pool I was in the hot TCA (RNA) fraction while the other two pools contained proportionately greater labelling in the lipid fractions. (It should be noted that Schmidt-Thannhauser fractionation in other experiments showed that all the label in the TCA fraction was associated with RNA, not with DNA.) The results here illustrated were consistent with other experiments which showed that the specific activity of RNA relative to the PFU titer was reasonably consistent ($0.64 - 1.5 \times 10^{-4}$). Thus it is clear that virus populations obtained from KB cell cultures contain particles of different grades of "completeness" and that this variability correlates consistently with variations in RNA contents.

TABLE I

Effect of Agar Inhibitor (AI) and Agar Overlay Medium on Plaque Production by Group B Arboviruses

	Source of Virus Stock	PFU/ml (log_{10}) Agar	Methylcellulose Untreated	Methylcellulose AI-treated*	% Residual PFU in AI-treated Virus
Dengue-2, New Guinea B	Mouse brain	6.5**	8.1	5.8	0.4
Dengue-2, Trin. 1751	Mouse brain	7.5**	9.0	8.2	13.0
Dengue-1, Hawaii	Mouse brain	7.0**	7.0	6.5	33.0
YF, 17D	Mouse brain	7.8**	8.1	7.9	63.0
MVE	KB cells	5.1	5.4	5.4	100.0
SLE	Mouse brain	10.0	10.0	10.0	100.0
JBE	KB cells	8.3	8.3	8.3	100.0

*Partially purified AI at a conc. known to inhibit 99% of PFU of Dengue-2 N.G.B. was added to each plating sample.

**These titers are based on estimated "plaque" counts. Plaques were so vague in outline that precise counting was impossible.

TABLE II

Fractionation of P^{32}-Labeled Dengue-2 Virus
Adsorbed on Goose RBC

Sample	Pool I	Pool II	Pool III
Original* HAU/ml	5×10^2	2.5×10^2	2.5×10^2
Original* PFU/ml	2×10^7	2.1×10^6	1.8×10^5
	CPM (%)	CPM (%)	CPM (%)
V-RBC, washed	5,334 (100)	1,804 (100)	1,524 (100)
Cold TCA	185 (4)	160 (10)	158 (10)
EtOH-ether	1,287 (28)	1,354 (82)	1,382 (87)
Hot TCA (RNA)	3,035 (67)	124 (7)	28 (2)**
Residues	58 (1)	11 (1)	16 (1)
All fractions	4,556 (100)	1,649 (100)	1,584 (100)
(% of V-RBC recovered)	(85.4)	(91.4)	(104)
CPM_{RNA}/PFU	1.5×10^{-4}	0.64×10^{-4}	(2.4×10^{-4})**

*Original: Pools before adsorption on RBC.

**Count too low to be reliable.

(3) Publications:

Schulze, I T , and Schlesinger, R.W. Plaque assay of dengue and other group B arthropod-borne viruses under methylcellulose overlay media. Virology 19, 40-48, 1963.

Schulze, I.T., and Schlesinger, R.W. (1963b). Inhibition of infectious and hemagglutinating properties of type 2 dengue virus by aqueous agar extracts. Virology 19, 49-57, 1963.

Stevens, T.M., and Schlesinger, R.W. (1963). Characterization of dengue virus. Fed. Proc. 22, 674.

Schulze, I.T. Reversible inhibition of type 2 dengue virus by agar polysaccharides. Virology (in press).

REPORT FROM THE INSTITUTE OF INTERNATIONAL MEDICINE, UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE

The early part of the year was devoted principally to the acquisition and training of additional laboratory personnel and to the preparation of reagents such as seed virus, antisera, hyperimmune sera, etc. During 1962, a large quantity of mosquitoes had been collected in the Lahore area of West Pakistan and was frozen for subsequent virus isolation processing. This material had been collected as a joint project of the University of Maryland School of Medicine and the Walter Reed Army Institute of Research and was arbitrarily divided by odd and even numbered lots between the two institutions for further study. During 1963, the University of Maryland processed 986 lots of this material, while the Walter Reed Army Institute of Research processed 901 lots. Each lot of mosquitoes was ground in a diluent containing bovine albumin and antibiotics and following centrifugation the supernate was inoculated intracerebrally into suckling white mice. Table 1 lists the materials tested by both institutions.

TABLE 1
Mosquito Material Collected in West Pakistan in 1962
and Processed for Virus Isolation

Species	Material tested at Univ. of Md.		Material tested at Walter Reed	
	No. pools	No. mosquitoes	No. pools	No. mosquitoes
Aedes albopictus	10	1114	11	1198
Aedes thomsoni	2	213	1	120
Aedes unilineatus	5	528	1	97
Aedes w-albus	5	549	5	439
Anopheles annularis	-	----	1	76
Anopheles culicifacies	26	2899	61	7013
Anopheles hyranus	2	230	3	341
Anopheles pulcherrimus	37	3842	74	7684
Anopheles stephensi	20	2228	60	6411
Anopheles subpictus	35	3642	47	4836
Culex bitaeniorhynchus	-	----	1	81
Culex epidesmus	1	120	1	42
Culex fatigans	18	1945	17	199
Culex tritaeniorhynchus complex	823	72283	617	60720
Mansonia uniformis	1	99	1	63
TOTALS	985	89692	901	89320

Three virus isolates were obtained from these materials. The isolate designations and pertinent collection data are as follows:

I-356 - from a pool of 100 female Culex tritaeniorhynchus complex taken in cattle biting collections at Shahzada village on August 3, 1962.

I-682 - from a pool of 100 female Culex tritaeniorhynchus complex taken in cattle biting collections at Shahzada village on August 16, 1962.

I-746 - from a pool of 100 female Culex tritaeniorhynchus complex taken in cattle biting collections at Kahna Kacha village on August 17, 1962.

Characterization and identification of the three virus isolates has been undertaken in our laboratories. Preliminary characterization by haemagglutination tests and neutralization tests indicate that all three isolates are West Nile or a closely related virus.

A smaller quantity of frozen mosquitoes collected in the Lahore area during 1963 also has been processed in our laboratories for virus isolation. No virus isolates have been obtained from this material to date.

Following demonstration of the natural infection of Culex tritaeniorhynchus complex mosquitoes, an effort was made to demonstrate experimentally their ability to transmit West Nile virus. Using a colonized strain of C. tritaeniorhynchus from Japan and a fully characterized strain of West Nile virus from Israel, it was demonstrated that this mosquito could readily transmit virus from viremic chicks to normal golden hamsters.

The mosquitoes from which the three virus isolates were recovered are members of a complex of several closely related species common to the Indo-Pakistan subcontinent of which C. tritaeniorhynchus and C. vishnui are members. Further virus isolation attempts on wild-caught C. tritaeniorhynchus clearly identified as distinct from other members of the complex are required. Should such further studies reveal that this species is commonly infected with West Nile virus, C. tritaeniorhynchus will have the unique distinction of serving as a principal vector of two common viruses, since its role as a vector of Japanese B encephalitis virus is already well established.

Parenthetically, it should be added that the distinction between Japanese B encephalitis and West Nile viruses in the Indo-Pakistan subcontinent appears to be more difficult than with virus isolates obtained from other geographic areas, since they cannot be readily differentiated by haemagglutination-inhibition procedures and differences in susceptibility of the golden hamster to the two viruses may not hold up for Indo-Pakistan strains.

Earlier studies on the sandfly fevers have been resumed. These studies had demonstrated the existence of 5 immunologically distinct strains of sandfly viruses. Preliminary haemagglutination-inhibition tests on a virus isolate (IP-6) originally obtained from a pool of female Phlebotomus collected in the Peshawar area of West Pakistan indicates that this virus is unrelated to the previously studied 5 types. Further studies are underway.

A number of species of mosquitoes have been colonized in Pakistan and subcolonized at the insectary in Baltimore to provide material for experimental transmission studies of ARBOviruses. These colonies include: Aedes albopictus, Aedes unilineatus, Armigeres subalbatus, and Culex fatigans. Efforts will be made during 1964 to colonize Culex tritaeniorhynchus and other members of the complex to which it belongs.

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

Plaque formation by type 1 dengue virus in chick embryo monolayer cultures (Dr. Eylar):

Primary cultures of chick embryo fibroblasts have been employed in an attempt to develop a readily available, uniformly acceptible cell culture system for the assay, cultivation and production of types 1 and 2 dengue viruses. Although in the early stages of development, this system appears to be promising provided that an enriched methyl cellulose overlay rather than the usual agar overlay is applied directly to the infected monolayers. First evidence of type 1 dengue virus (mouse adapted Hawaiian strain, mouse passage 122 and the attenuated MD-1 strain, mouse passage 34) occurred 4 days post-infection with the appearance of pinpoint plaques. Plaque size increased slightly through day 7 with a maximum plaque diameter of 1 mm. Comparative titrations in vivo and in vitro indicated that the ratio of suckling mouse LD_{50} to plaque count was approximately 2/1.

Most attempts to demonstrate plaque formation in this cell system by the New Guinea B strain of type 2 dengue virus were unsuccessful. In some instances, however, plaques were observed but these were small and indistinct.

The influence of "accessory factor" on West Nile virus neutralization (Mr. Chandler, Dr. Eylar):

West Nile virus neutralization kinetics in a chick embryo fibroblast culture assay have been employed in the study of the level and nature of "accessory factor" in various mammalian sera.

"Accessory factor" obtained from individual human donors varied remarkedly in "accessory factor" activity. Although some individual human sera contained little or no detectable "accessory factor", when mixed with sera known to contain moderate to high levels of "accessory factor", neutralization rates increased to a level in excess of that obtained with individual sera. The neutralization rate constant (K) for the pooled sera was in excess of the K value obtained by averaging results from individual sera, suggesting that "accessory factor" may contain more than one limiting component.

Titrations of pooled "accessory factor" suggested that the arbitrary selection of 25% "accessory factor" was not necessarily an optimal level and that slight changes in the "accessory factor" concentration added to the neutralization mixture could significantly alter the rate of virus neutralization.

Incubation of "accessory factor" with specific precipitates removed essentially all of the "accessory factor" activity and the K values obtained were reduced to the level of those obtained with heat-inactivated sera. These latter results suggest that complement or a complement-like component(s) may be important to "accessory factor" activity.

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

Immunological Studies on Arboviruses*

Dr. W.P. Allen continues his studies on the immunological overlap among the arboviruses. In recent work, protection of mice vaccinated with Group A arboviruses against challenges with heterotypic viruses was investigated to determine whether resistance is fully manifested prior to challenge, or if such resistance develops as a result of the challenge stimulus. Cortisone acetate was administered intramuscularly in 4 daily doses of 5 mg each, commencing the day before inoculation of the challenge virus to suppress the immune response to the challenge stimulus. This treatment inhibited protection against Venezuelan equine encephalitis virus (VEE) in mice previously vaccinated with Sindbis or Semliki Forest (SF) viruses. Protection against SF virus was partially inhibited by cortisone in mice vaccinated with VEE or Sindbis viruses. Homologous protection in immune mice was essentially unaltered by cortisone treatment. The data suggest that for certain cross-protection systems resistance to heterotypic viruses is manifested in the vaccinated animal as a result of the challenge stimulus and is not fully developed prior to the challenge.

Neutralizing antibodies to viruses against which the mice were vaccinated were present prior to challenge and probably accounted for the homotypic resistance, but neutralizing antibodies to heterotypic viruses were not detectable at that time. Normally, antibodies to the challenge virus appear in the serum within 7 days after the challenge is administered. It has not been determined yet whether cortisone suppresses the formation of or delays the appearance of these antibodies.

*In conducting the research reported herein, the investigators adhered to 'Principles of Laboratory Animal Care' as established by the National Society for Medical Research.

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

The priority application of the Arbovirus Laboratory resources has been directed toward continued support of the field and laboratory investigations of EEE surveillance in South Georgia, dengue in the Caribbean, and continued search for arbovirus activity in the Everglades and adjacent areas of southern Florida. Description of the initiating problems and progress has been reported in previous issues of the Information Exchange.

In the South Georgia EEE surveillance, 60,000 mosquitoes collected between August first and mid-October have now been processed and yielded at least 165 strains of virus. Preliminary characterization and identification distributes the isolates by type and mosquito species of origin according to the following tabulation.

Tentative Identification of 1963 Mosquito Isolates from South Georgia*

Virus	Mosquito Species	No. of Isolates
EEE	Culiseta melanura	34
	Aedes atlanticus-tormentor	1
	Culex nigripalpus	1
WEE**	Culiseta melanura	14
Calif. Group	Aedes atlanticus-tormentor	43
	Anopheles crucians	1
Tensaw	Psorophora confinnis	16
	Anopheles crucians	19
	Aedes atlanticus-tormentor	2
	Aedes mitchellae	3
Hart Park	Culiseta melanura	30

*Identification by complement-fixation of crude antigens with known antisera.
**All WEE isolates identified by neutralization tests.

Final analysis has been made of the laboratory evidence of EEE virus activity and disease in equines which initially focussed concern on the South Georgia area as a potential epidemic area.

No. of Specimens	Type of Specimens	No. Positive for EEE	No. Presumptive for EEE
26	Brains	16 virus isol.	
19	Sera	2 (CF+)	8 (CF-)

Although continued efforts to establish an etiological virus from substantial numbers of mosquito and acute human serum specimens collected in Jamaica and Puerto Rico in the continuing Caribbean dengue epidemic have so far failed to yield a usable strain for testing, serological studies of paired and serial sera from human cases examined in Jamaica, Puerto Rico, Virgin Islands, and USA have been HI and CF tested against dengue types I, II, III, and IV, YF, Ilheus, SLE, and MVE antigens. Analysis of a large amount of accumulated data indicates that yellow fever infection or vaccination is of negligible importance in the sera showing broad Group B reactions and that although there has been rather frequent exposure of the Jamaican population to Group B viruses, the younger population of the Guaynabo study area of San Juan, Puerto Rico, does not appear to have been exposed to a Group B virus, including dengue, since the epidemic reported in 1944. Further statistical analysis of the HI and CF results indicate that the etiological agent is not dengue type I, II, or IV, but most closely related to dengue III.

Intensive long-term search for arbovirus activity in the Everglades habitat of south Florida, following the finding of VEE virus antibodies in the Big Cypress Seminole Indians in 1960, has continued with mosquito and bird collections in the Big Cypress area in 1961 and 1962. These collections were extended deep into the Everglades with assistance of the U.S. Department of the Interior National Park Service in 1963.

Collections were carried out in May-June, mid-July, and mid-September. At least three strains of Group A arboviruses have been isolated, all from Culex (Melanoconium) species.

REPORT FROM DR. MAURICE PROVOST AND DR. RICHARD DOW
FLORIDA STATE BOARD OF HEALTH ENTOMOLOGICAL RESEARCH STATION
VERO BEACH, FLORIDA

A laboratory colony of Culex nigripalpus started by Mr. James S. Haeger now contains over 2300 adults of the F_4 generation. Attempts at copulation observed in an indoor cage at light intensities between 0.45 and 0.08 log lux had not resulted in fertile eggs so that the problem was to induce normal mating under laboratory conditions. The solution was found by introducing mosquitoes of other species (Aedes taeniorhynchus and Culex quinquefasciatus) which would swarm and mate at light intensities suitable for similar behavior in C. nigripalpus. There are no observations to show whether the successful matings in C. nigripalpus were stimulated by the mating, swarming, or other flight activity of the introduced species. The present stock of the colony is derived from 4 egg rafts, which, laid by wild females, started the parent generation, and 5 F_1, 2 F_2, 16 F_3, and 16 F_4 egg rafts. Some mating has taken place without stimulation in adults of the F_4 generation (in 22 of 100 females the spermathecae contained sperm), but it is not yet known whether Mr. Haeger can select a strain which will continue to mate successfully in a cage.

Studies of C. nigripalpus made at Vero Beach in the winter of 1962-63 suggested that in spite of continued capture in bait traps, the females were not laying eggs and were perhaps using their blood meals as a source of energy for daily activity. During the current winter, in addition to the operation of bait, suction, and light traps, the oviposition of C. nigripalpus has been sampled by means of watertight redwood boxes filled with an oak leaf infusion and exposed under a small shelter. The technique was developed from a study of oviposition behavior begun last summer. Observations to date show that there is normal interruption of activity by cold weather but give no indication of a winter diapause expressed by cessation of egg laying.

REPORT FROM DR. JAMES O. BOND
FLORIDA STATE BOARD OF HEALTH ENCEPHALITIS RESEARCH CENTER
TAMPA, FLORIDA

Initiated as a temporary field station during the 1962 epidemic of SLE, the Tampa Bay Regional Encephalitis Laboratory assumed more permanent status on December 1, 1962, following the award of a five-year grant from the National Institutes of Health (NIH) to the State Board of Health (SBH). The laboratory was

was established in a building provided by the Southwest Florida Tuberculosis Hospital on its grounds in Tampa. Later in 1963, the $186,000 NIH grant was supplemented by $100,000 appropriated by the Florida State Legislature for research, surveillance, and control of encephalitis. A portion of the state money was allocated to the Tampa Laboratory which was then officially named the Encephalitis Research Center (ERC).

The center has a staff of 24, directed by Dr. James O. Bond, who is immediately responsible to the State Health Officer. The activities of the center are coordinated with other statewide research and surveillance activities in encephalitis by Assistant State Health Officer, Dr. Albert V. Hardy. The various scientific disciplines in the ERC maintain close consulting relationships with their appropriate bureaus in the SBH. During 1963, the following professional persons were responsible for the sectional activities in the Research Center: Dr. Donald T. Quick, EIS Officer from the CDC, USPHS, Epidemiology; Dr. Arthur L. Lewis, Virology; Dr. William L. Jennings, Biology; and Mr. Doyle J. Taylor, Entomology. The Research Center maintains close cooperative relationships with the County Health Departments and the County Mosquito Control Districts in Hillsborough, Manatee, Pinellas, and Sarasota Counties. The Arbovirus Laboratories of the CDC in Atlanta, Georgia, and the University of Pittsburgh Graduate School of Public Health are utilized for special reference and consultative assistance.

A nine-member Advisory Committee on Encephalitis was appointed from a panel of nationwide experts to advise the State Health Officer on statewide research, surveillance and control activities, including those carried out in the ERC.

The scientific activities of the Encephalitis Research Center in 1963, although planned as a team effort, will be presented as a report from each section.

The establishment and maintenance of a surveillance and reporting system for all central nervous system viral disease in the Tampa Bay area resulted in a high index of suspicion among medical and public health personnel. Of 301 suspected viral infections of the central nervous system in humans brought to the attention of the ERC, 82 were finally appraised as representing cases of infectious encephalitis, aseptic meningitis, or paralytic disease. There were no acute cases of St. Louis encephalitis (SLE) with laboratory confirmation in the four-county area during 1963, although one was reported

on the basis of clinical findings. One California virus infection was confirmed, the second reported occurrence of encephalitis following infection with this virus in the United States. Of the remaining cases in which a definite etiology was established, two were from measles, 13 from mumps, four influenza, two poliomyelitis, and one leptospirosis. In 64 per cent of the 82 cases, no etiological relationships could be established.

Three major serologic surveys for inapparent SLE infection were carried out or completed during 1963. These involved 3000 individuals in areas of Bradenton, Sarasota, Clearwater, Tampa, and rural Hillsborough County. Prevalence rates for preceding inapparent infections with SLE in these areas were as follows: Clearwater 8.1 per cent, Tampa 4.1 per cent, Bradenton-Sarasota 3.4 per cent. A small group of the Hillsborough County residents were rebled in December of 1963 and there was no evidence in their sera that any transmission of SLE virus to humans occurred during the summer months. Small samples of the 3000 survey individuals were also examined for prevalence of antibodies against California and Tensaw viruses. It was found that from one to six per cent of these had previous infection with California virus and from one to five per cent with the Tensaw virus. There was no evidence of inapparent infection with either of these viruses during the summer period of 1963.

Studies on antibody response following acute clinical SLE were carried out on selected patients from the 1959-1961 and 1962 epidemics. Although not complete, early findings from these studies indicate that the CF antibodies are of very short duration, usually persisting only a few months. The HAI antibodies fell to very low levels within the first two years and were below detectable levels at the end of the third year. Serum neutralizing antibodies, on the other hand, have been shown to persist for the full three years of the follow-up studies.

The Entomological Section maintained chick baited traps on a semiweekly operation at 12 stations from January to April, and at 28 stations from May to December 1. Six of these (three in mosquito control areas), are key stations where thermographs and rain gauges are operated and are adjacent to six truck trap runs. As of December 31, 72,746 mosquitoes were captured by bait traps and submitted in 2592 pools for virologic testing. From eight pools of *Aedes infirmatus* mosquitoes, isolations were made of a viral agent

later identified as closely related to the Trivitattus virus which is a member of the California group. A ninth isolation, from Aedes taeniorhynchus mosquitoes, was identified as Tensaw virus, a member of the Bunyamwera group. The California group viruses were isolated from mosquitoes trapped in Pinellas, Hillsborough, and Manatee Counties, and the Tensaw virus was obtained from Hillsborough County. In 890 pools prepared from 47,093 Culex nigripalpus mosquitoes collected in bait traps, no viral isolations were made.

Truck trap collections were used to establish the densities of various mosquito species in the controlled and uncontrolled areas, and to determine the percentage of gravid and parous female mosquitoes of the sub-genus Culex. A total of 61,759 mosquitoes were collected in the truck traps, of which 13,725 were in controlled areas, and 48,034 in uncontrolled areas. The pattern of consistently lower numbers of mosquitoes collected by the truck traps in controlled areas was found in each of the three counties. In Hillsborough and Pinellas County, the percentages of parous or gravid female mosquitoes were consistently and significantly lower in the controlled compared to the uncontrolled.

Meterological observations on temperature and rainfall were compared to the mosquito densities in each of the collection sites. Culex nigripalpus densities were unusually low in all three counties and were particularly low in Pinellas County where intensive adulticide efforts were carried out from early spring through late October. In all three counties, there were small amounts of rainfall in the spring and early summer, and this combined with the low water table made conditions particularly unfavorable for early C. nigripalpus development. Late in the fall there was a rise in C. nigripalpus density, but apparently too late for any significant effect on SLE transmission. The relationship of rainfall to C. nigripalpus ovi position and flight activities, and the relation of sugar meals to longevity of the mosquito were important biological observations contributed by the Entomological Research Center in Vero Beach, based upon data collected in cooperation with the Encephalitis Research Center.

Virus activity in vertebrates was measured by serological and virological examinations of sentinel chickens, nestling wild birds, trapped wild mammals, and sick birds and mammals collected from the epidemic area. During the period March through November, 794 sentinel chickens were exposed in standardized traps during ten separate three week periods.

Sixteen of these chickens had low titers of HAI antibodies against SLE, and all were negative against EE. The significance of these low HAI titers cannot be assessed until neutralization antibody studies are performed on these sera. It is presumed, however, that they likely represent non-specific inhibitors in the sera rather than SLE antibodies. Three of 376 nestling rookery species had low SLE-HAI antibody titers, and three of 390 urban nestling species had similar titers. All three of these were from 333 nestling doves collected in the epidemic area of St. Petersburg. Two of 42 marsh and shore bird nestlings, and one of 324 small mammals also exhibited low SLE-HAI antibody titers. Of the titers observed, only one, a titer of 1:160 in a nestling redwing blackbird, was considered to be of definite significance, representing recent infection with SLE.

Laboratory infection experiments carried out with four day and four week old chickens indicated that viremia and high antibody titers are rare in older chickens, but relatively common in the young. Similar infection experiments with doves indicated that they respond with higher antibody levels and a greater proportion have prolonged viremias, compared to chickens.

Special ornithological observations were carried out by Dr. Glen Woolfenden of the University of South Florida with the assistance of graduate students. The avian populations in selected areas in the three counties were quantitated using both a strip census and a nesting census technique. Using the former technique, the densities of total birds per census mile were found to vary from 160 in Tampa to 189 in St. Petersburg. Seventy-four species were recorded in all, but 90 per cent or more of the individuals could be accounted for by only 12 species. There were significant differences in the relative densities of these 12 species in the four study areas. Comparative observations for time of year, technique, and area were made in both St. Petersburg and Tampa in 1962 and 1963. Remarkably little variation was found in the relative density of birds during the two periods of time. A special area census of nesting birds was carried out during the spring and summer of 1963 in three plots in St. Petersburg. The housesparrow was most frequently encountered, followed by the mourning dove. A close association was found between the density of mourning doves and the type of vegetation. High densities were found in pine plot areas, smaller densities in oak plots and very low densities in plots in which there was less than one per cent standing vegetation coverage.

Following a period of renovation of space and training of personnel, the virology laboratory began functioning on a routine basis in April of 1963. Through December, 2592 pools of mosquitoes have been processed for virological specimens in suckling mice, and 5331 serum specimens examined for the presence of antibody against various arboviral agents. In all, nine viral isolations were obtained as previously described. Following a third suckling mouse passage, these viral agents were shown by the ERC virology laboratory to be eastern, western, or St. Louis encephalitis virus by the complement fixation and neutralization screening tests. None of the isolates produced a hemagglutinin and all were shown sensitive to sodium desoxycholate. They were then forwarded to the University of Pittsburgh or the CDC virological laboratories for final identification. Eight of the isolates were demonstrated to belong to the California group by cross-neutralization, CF and HAI tests. They are most closely related to the trivitattus agent, first isolated in North Dakota in 1948. The ninth agent was shown to belong to the Bunyamwera group by the ERC virology laboratory and confirmed at both CDC and the University of Pittsburgh. Other cooperative and comparative serological studies were carried out with CDC and the University of Pittsburgh to determine the sensitivity and specificity of the different arbovirus antigens used in the ERC battery.

In cooperation with the virology section of the SBH laboratories in Jacksonville, additional virological diagnostic studies were carried out on human specimens from the four county area in which there was no evidence of arbovirus activity. Of 133 stool specimens tested in Jacksonville, nine enterovirus isolations were made. Two of these were poliomyelitis, and the remainder were Echo-6 and Echo-7. Attempts to isolate virus from 55 cerebrospinal fluids were made; all were negative. In addition, serological examinations were performed for mumps in 45 individuals, 13 cases were confirmed; leptospirosis, 73 examined, one case confirmed; LCM 36 examined, none confirmed; poliomyelitis 47 examined, two confirmed; herpes simplex 16 examined, none confirmed; and measles two examined, none confirmed.

Five students received training experience in the ERC during 1963. Two were undergraduates in the field of biology and laboratory technology, and three were graduate students, one each from medicine, entomology, and biometry. A Public Health Veterinarian and a Public Health Physician also spent some time in residence at the Center for training experience in

arbovirus epidemiology. Publications: Tampa Bay Area Arbovirus Investigations, 1959-1961. Florida State Board of Health Monograph #5, November, 1963. This monograph is available free of charge upon written request to the Division of Health Education, Florida State Board of Health, Post Office Box 210, Jacksonville, Florida.

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

Encephalitis and aseptic meningitis caused by a group B virus closely related to St. Louis encephalitis virus has been observed to occur sporadically in the South Florida area. In selecting a sentinel animal to provide a continuing check on the activity of SLE virus in the Miami area, it was found that a large percentage of common pigeons appeared, on the basis of HI titer, to have been infected naturally in the Miami area. Pigeons were chosen as a sentinel animal because the local pigeons are true lifelong residents of this area and show no characteristics of migration. As a consequence of infection by the subcutaneous route with SLE virus, serologically negative pigeons developed hemagglutination inhibition titers of 1/320 or more with as little as 10 LD_{50} of virus. Serologically negative pigeons are now being maintained in selected sites around the Miami area and are being bled at three week intervals. No conversions have been observed in these pigeons during 1963 and early 1964 but also cases of encephalitis or aseptic meningitis due to St. Louis encephalitis virus have not been observed during the same period in the residents of Miami.

A group of 136 Seminole Indians (Miccosukee tribe) located within 40 miles of Miami are under study for evidence of past or current arbovirus infection. These Indians were bled by us as part of a comprehensive health survey of the tribe carried out by the Department of Research and Epidemiology of the Dade County Public Health Department. Tests available thus far indicate SLE HI titers of 1:20 to 1:40 or over in only four of 67 tested.

REPORT FROM DR. CARLOS CAMPILLO-SAINZ,
DR. JULIO DE MUCHA-MACIAS, AND Q.B.P RAUL RUBIO BRITO,
INSTITUTO NACIONAL DE VIROLOGIA
MEXICO 7, D.F.

Report of Arbovirus Studies in Mexico

The present report deals with the entomological surveys and virus isolation attempts from arthropods which have been undertaken in the Instituto Nacional de Virologia as part of its research program on arboviruses. So far, a total of 7,841 mosquitoes distributed in 154 pools have been studied. Virus isolations have been attempted in suckling mice by the intracerebral route.

In the accompanying table (see next page), the pertinent data obtained in Coatetelco (State of Morelos) and Tlacotalpan (State of Veracruz) are given.

VIRUS ISOLATION ATTEMPTS FROM MOSQUITOES COLLECTED AT COATETELCO, STATE OF MORELOS, AND TLACOTALPAN, STATE OF VERACRUZ, 1963

LOCALITY	SPECIES	FEMALE MOSQUITO SPECIMENS	NO. OF POOLS	RESULTS		
				NEGATIVE	INCERTAINES	POSITIVES
Coatetelco	A. pseudopunctipennis	50	1	1	0	0
	C. coronator	50	1	1	0	0
	C. iolambdis	50	1	1	0	0
Subtotals		150	3	3	0	0
Tlacotalpan	A. pseudopunctipennis	250	5	5	0	0
	A. scapularis	51	2	1	1	0
	A. albimanus	722	14	10	3	1*
	A. vestitipennis	167	4	3	1	0
	C. coronator	100	2	2	0	0
	C. iolambdis	200	4	4	0	0
	C. nigripalpus	524	11	8	3	0
	C. quinquefasciatus	100	2	2	0	0
	M. indubitans	180	4	4	0	0
	M. nigricans	5	1	1	0	0
	M. titillans	5385	101	64	37	0
	P. lutzi	7	1	0	1	0
Subtotals		7691	151	104	46	1
Grand Totals		7841	154	107	46**	1

* This agent has been passed nine times in suckling mice and its identification is underway.

**Pools not defined either positive or negative have been reinoculated. Results will be given in the near future.

REPORT FROM DRS. K.M. JOHNSON, R.M. MACKENZIE, AND M.L. KUNS MIDDLE AMERICA RESEARCH UNIT, PANAMA

Epidemic Hemorrhagic Fever in Bolivia

During 1963, three major field expeditions were mounted to investigate this problem in the eastern prairie Province of Beni. Exploratory observations by Drs. Beye, Kuns, and Mackenzie during March and April indicated that the town of San Joaquin was the principal focus of human disease. With the help of the Bolivian Ministry of Health and Department of Rivers and Lakes, and with logistic support of U.S. Military Groups in Panama and La Paz, intensive examination of the problem was begun in mid-May. A team composed of Drs. Kuns, Mackenzie, and Johnson from MARU, Dr. Conrad Yunker of the Rocky Mountain Laboratory, Dr. Luis Valverde and Dr. Hugo Garron of the Bolivian Ministry of Health and Dr. Jack Woodall from the East African Virus Research Institute, Entebbe, Uganda (Dr. Woodall was sponsored by The Rockefeller Foundation), ably supported by Mr. Gustavo Justines and Mr. Angel Munoz of MARU, Miss Rose Navarro, R.N., Peace Corps, Bolivia, and doctors and hospital corpsmen from the U.S. Army Southern Command, Panama and Bolivia, worked for eight weeks on virologic, epidemiologic, ecologic, and clinical facets of the disease. During this interval, the etiologic virus was isolated, the clinical disease was partially delineated, and much information concerning the epidemiology and ecology of the disease in and about San Joaquin was obtained. Human, mammalian, and arthropod specimens for virus isolation were processed and several thousand others were obtained for future examination. This expedition was terminated in early July. At about this time, Dr. Mackenzie, Dr. Johnson, and Mr. Munoz contracted the disease, followed shortly by Sgt. Robert Lowery, U.S. Army. All four made complete recoveries in Panama.

A final trip was made in August-September to extend earlier observations and to focus specifically on the problem of potential reservoir hosts and vectors of the disease. During this trip, we were fortunate to have the counsel of Dr. Harald Johnson, Rockefeller Foundation, Berkeley, California.

Interim Progress

A major problem was, and is, protection of personnel from the disease, both in the field and in the laboratory. Plastic isolettes were used for all manipulations of potentially infected specimens in San Joaquin, repellents and repellent-

treated clothing were systematically used, and all workers were given "hyperimmune" gamma globulin obtained from persons with at least a previous history of hemorrhagic fever. (This was prepared through the courtesy of Dr. Geoffrey Edsall and staff at Massachusetts State Public Health Laboratories.) Nevertheless, as noted, four cases of hemorrhagic fever occurred during a period of about 110 man weeks of exposure.

Even more startling was the occurrence of confirmed hemorrhagic fever in two wives of personnel under circumstances which strongly suggested that transmission was human to human, and had occurred in the hospital. On the basis of these considerations, a special laboratory was constructed at MARU to provide protection against aerosols for other persons in the building. This laboratory was activated in December and all work on the hemorrhagic fever virus is now conducted by four immune MARU staff members.

The virus

The original isolate was obtained from the spleen of a two-year-old boy who died of hemorrhagic fever on May 18, 1963. Designated strain Carvallo, it currently is under intensive study as the provisional prototype. The virus is pathogenic for both infant mice and hamsters by intracerebral and intraperitoneal routes. Average survival time is somewhat shorter in hamsters (about 12 days) than mice (about 15 days) and there is a better correlation between ID_{50} and LD_{50} in hamsters than mice. The agent is not significantly pathogenic for either adult mice or hamsters. The virus was completely inactivated by exposure to 10% chloroform for 10 minutes at room temperature.

Complement fixation studies linking Carvallo virus and Junin and Tacaribe viruses have been reported by Dr. Wiebenga in previous issues of the exchange. Preliminary results (see page) indicate that the three viruses are completely distinct by neutralization test. Crude alkaline extracted CF antigens have readily been prepared from infected hamster and mouse tissues. Table 1 presents results obtained in one hamster experiment following IC-IP inoculation of about $10^3 ID_{50}$. Brain antigens are now being used for all routine preliminary identification of isolates and for reidentifying virus employed in laboratory experiments.

Preliminary tissue culture studies resulted in selection of human embryonic lung fibroblasts (strain WI-26) as cell system of choice. Rather nondescript CPE is regularly produced

by Carvallo virus in these cultures and, when rotated on a roller drum, complete degeneration of cell sheets is achieved. The system seems more sentitive at 34° than 37°C. Cultures do not require refeeding (Eagles - 2% calf serum) at more than 5-7 day intervals and endpoints are generally reached after 10-12 days. Differential sensitivity of WI-26, infant hamster and infant mouse for virus isolation is included in Table II. The relative inefficiency of the tissue culture system should prove to be partially compensated by the larger volume of inoculum that can be employed.

Other Isolates

Table II summarizes the status of agents originally recovered in San Joaquin. All of these strains have been grouped with Carvallo virus by CF test. None have yet been identified by neutralization test. The recovery of two strains from *Caolmys callosus*, however, provides the first clue for the existence of a non-human virus cycle in nature.

Epidemiology and Ecology

Serological testing of 1963 Bolivia sera has been limited to the complement fixation test (CFT). Such testing has been directed toward:

a. Confirming the diagnosis of Bolivian hemorrhagic fever (BHF) on hospitalized cases in which clinical findings were recorded.

b. Determining the levels of complement fixing (CF) antibody in relation to time subsequent to onset of clinical BHF.

c. Defining the epidemic curve of clinical BHF during 1963.

d. Estimating the frequency of apparent and inapparent BHF infection among San Joaquin, Bolivia, population.

We have tested 210 convalescent phase serum specimens from 73 serologically confirmed cases. CF antibody appeared frequently 10 to 20 days after onset but three individuals were still negatives or had titer of 1:2 during the 30-40 day interval. The maximum mean titer (1:128) occurred between 40 and 50 days after onset of illness. The mean titer dropped to a level of about 1:16 after 160 days, but on one occasion CF antibodies were undetectable at that time. In this instance

no antibodies were detected when antigen was increased from 2 to 8 units. One other confirmed case was CFT negative after 190 days. Such observations place definite limitations on the CFT as an epidemiologic tool in BHF.

We have studied serially obtained sera from 170 hospitalized cases with onsets from March through August. Results suggest that serologically confirmable BHF cases were admitted at a steady rate of 30 to 35 per month with the exception of March and June when an estimated 57 and 56 cases were admitted. San Joaquin hospital records do not suggest any other epidemic peaks during the year. The month in 1963 of lowest disease activity has not yet been determined. The overall case fatality rate among hospitalized cases appears to have been about 20%. Studies on about 300 randomly selected human sera from the San Joaquin population are still in progress.

Human cases have been confined to villages and isolated settlements located in the tropical semi-deciduous forests. These forests form a well-defined vegetation type occupying sites with relatively rich soils and sufficient elevation to escape the annual floods. No human BHF cases have been reported from villages and settlements located in the swamp forests which line the banks of rivers and streams. Infestation of houses by the rodent, Calomys callosus, has been observed in each of five villages studied where human BHF cases have occurred. This rodent was collected in only one of four nearby villages where BHF cases have not been reported. Ectoparasites collected from Calomys callosus in San Joaquin, and identified by Drs Conrad Yunker and Glen Kohls, include larval hard ticks (Amblyomma sp.), four species of chiggers (Trombiculidae), and the mites Ornithonyssus bacoti and Haemolaelaps sp. (H. Glasgowi complex).

During the period May 15 through August 31, 1963, approximately 90% of the confirmed BHF cases in San Joaquin were in persons residing in the southern half of the village. The northern perimeter of the town borders grassland habitats while remnants of the original semi-deciduous forest form the southern periphery. The sylvan rodent, Proechimys guyannensis, was occasionally trapped in the southern half of the village but has not been collected in the northern half. The rodents, Calomys callosus, Oryzomys bicolor, and Holochilus brasiliensis, were distributed throughout the village. Preliminary serological studies using the complement fixation test indicate that both Calomys callosus and Proechimys guyannensis are occasionally infected with BHF virus.

Table I

CF Antigens in Infant Hamster Tissues

Tissue	Reciprocal CF Titer, Indicated Post-Inoculation Day				
	4	5	6	7	8
Brain	8	8	16	32	32
Spleen	2	4	8	128	128
Liver	2	2	4	16	128

20% tissue suspensions in borate saline pH9 extracted overnight at 40° C.

Table II

Agents Tentatively Identified as HF Virus

SOURCE	SPECIMEN	ISOLATION SYSTEM	REISOLATION (Log_{10} ID_{50}/g.or ml)		
			Hamster	Mouse	WI-26
Human:					
#1 (Carvallo)	Spleen	Hamster	6.2	4.3	4.7
#2	Spleen	Mouse	4.1	3.6	3.2
#3	Buffy coat	Mouse	3.2	<2.5	2.5
#4	Spleen	Mouse	Pending		
#5	Whole blood	Hamster	"		
Calomys callosus					
#1	Whole blood	Mouse	5.0	3.7	3.7
#2	Brain	Mouse	Pending		

REPORT FROM DR. NED WIEBENGA
LABORATORY OF TROPICAL VIROLOGY
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
BETHESDA, MARYLAND

Bolivian Hemorrhagic Fever

In the previous Infoexchange, we reported the relationship of BHF to Junin and Tacaribe viruses by the complement fixation test. In the same issue, Dr. Downs reported plaque production by Junin and Tacaribe viruses and serum neutralization tests in primary rhesus monkey kidney cell cultures.

In similar studies here BHF virus produced plaques like those of Junin and Tacaribe viruses in primary rhesus MK. All 3 viruses produced similar plaques in MA 104 monkey kidney and MA 111 rabbit kidney, continuous cell lines developed and provided by Mr. M. Vincent, Microbiological Associates, Inc. Studies for detailed comparison of these TC systems are still in progress, but comparable neutralization results were obtained in each.

The accompanying table is a summary of replicate tests,using hyperimmune ascitic fluids and rabbit serum. Cross neutralization tests showed plaque reduction by homologous antibody with negligible cross reactions. Thus, while BHF virus was related to Tacaribe and Junin by complement fixation tests, present studies indicate that it is distinct by serum neutralization.

BHF Virus
Plaque Reduction by Serum Neutralization

Virus	Titer $\log_{10}$ PFU/.2 ml	Antibody: log Neut. Index BHF	Junin	Tacaribe
BHF	5.8	3.5	0	0
Junin	6.5	0.5	4.5	1.0
Tacaribe	7.2	0.5	0	3.5

The complement fixation tests on human sera from suspected cases of Bolivian hemorrhagic fever, 1963, have been completed. Of 54 individuals from whom serial specimens could be examined, 38 (70%) demonstrated serological conversion from $<$1:2 to titers between 1:4 and 1:128. Twenty-two (58%) of those had titers of 1:32 or more. Sixteen (30%) remained negative. However, Dr. Mackenzie (MARU) reports 52/55 (95%) serological conversion when additional specimens were collected and tested with similar CF antigens.

REPORT FROM BELEM VIRUS LABORATORY, BELEM, BRAZIL

The virus of EEE reappeared in the Belem study area in 1963 (see graph) after an apparent absence of two years, from January 1961 through December 1962. Isolations were made over a period of 9 months (January through September 1963) from sentinel mice, monkeys, and chickens and from mosquitoes associated with them or caught on human bait. Two possible isolations from birds, and one each from Proechimys, Oryzomys, and Marmosa were not confirmed by reisolation. HI antibody studies on bird and animal sera collected during this period failed to show evidence of previous epizootic infection with this virus. These observations indicate that the natural vertebrate host for EEE in this area was not detected by the surveillance methods used.

In an attempt to gain some idea of susceptibility, 6 wild-caught animals were inoculated subcutaneously with 10,000 to 100,000 LD_{50} (for infant mice) of a recently isolated strain of EEE virus. One of two Marmosa murina circulated virus for 4 days as shown by infection of infant mice following inoculation with sera from this animal. Two Proechimys guyanensis and two Oryzomys goeldi failed to circulate virus in this experiment. The pre- and post-serology is not yet complete on all animals, but at 3 weeks post-inoculation, the positive Marmosa had an HI titer of 1-20 and NT of 3 logs.

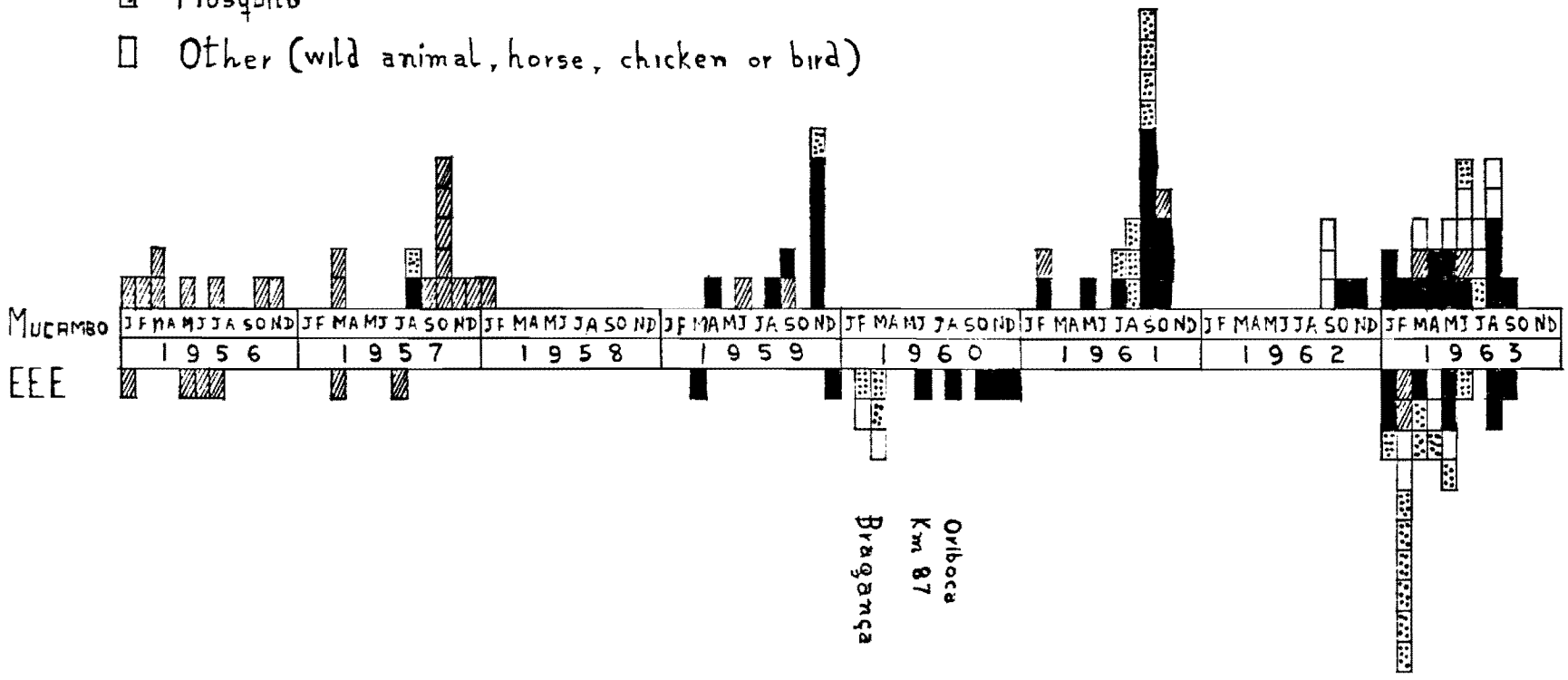
Mucambo virus (formerly called VEE in Belem) also was isolated in 1963 from sentinels and mosquitoes and confirmed from wild rodents and marsupials as well. HI and NT results on sera of captured rodents also indicate that Mucambo was active in the study area during the 13 months following September 1962.

A new approach for epidemiological investigations in the animal recapture program is being developed. It had been planned to tag rats of the genus Proechimys with radioactive substances in order to follow them to their places of abode for information about their lives between captures and to collect the fleas, ticks, and other insect parasites from their natural resting places. Due to difficulty encountered

in arranging the necessary equipment, a simpler method was adopted. One end of a spool of nylon thread is attached to the rodent, either by tieing the thread around the waist of the animal, or securing it to a ladies watch strap, which is fastened around the lower abdomen. When it was desired to retrieve the animal from the burrow, the latter method is used. Routinely, the string is tied on without a strap. By playing the string back and forth after the animal has gone under or into a fallen tree or ground tunnel, the animal will usually continue on his way if the spot is just a refuge. After reaching home, however, he will not proceed further. The string is then cut at the entrance and a stick with the pertinent data is attached to the string. The string is left just as the rat unwound it from the spool. This serves to mark the runway. Although frequently no visible trail is apparent, the same animal usually follows the identical route when he is liberated after subsequent recaptures. In some instances a part of the same trail may be used by several individuals that live in different locations.

Figure 1

Isolations of Mucambo and EEE Viruses in Belém Study Areas 1956–1963 (Utinga-IAN forests except as marked)



REPORT FROM DR. LOUIS S. GRANT
MICROBIOLOGY DEPARTMENT, UNIVERSITY OF THE WEST INDIES, KINGSTON, JAMAICA

Dengue-like Epidemic in Jamaica:

An epidemic of a dengue-like illness started in Jamaica in June 1963. At the end of 1963, approximately 1,500 cases had been reported to the Ministry of Health. Cases were reported mainly from Kingston and St. Andrew, but a number of cases were reported from other parishes. Symptoms and signs were fever, headaches, retroorbital pain, generalized body pains, rash on chest and back and arms, generalized lymphadenopathy leucopenia with relative lymphocytosis and atypical mononuclear cells, occasional gastro-intestinal symptoms (nausea, vomiting, or diarrhea).

Incidence of adult *Aedes aegypti* in houses in areas of high prevalence range from 17% to 54% and incidence of *Aedes aegypti* larvae in houses in Kingston and St. Andrew ranged from 5% to 43%.

Cases of the dengue-like illness have occurred in adults, mainly, but there have also been some cases in school children, nearly one thousand acute and convalescent serum specimens have been referred to the Department of Microbiology, University of the West Indies. Mosquitoes have also been collected in the city of Kingston by Dr. Sudia of the Communicable Disease Center and others, and these have been used for attempted virus isolations.

Technical and professional advisory assistance has been rendered by Dr. Telford H. Work and others at the Communicable Disease Center, Atlanta, Georgia, the Rockefeller Foundation Virus Laboratory, New York, the Trinidad Regional Virus Laboratory, Dr. W.McD. Hammon's laboratory at the University of Pittsburgh, Dr. Walter Schlesinger of Rutger's University, Dr. Morris Schaeffer of the New York City Bureau of Laboratories, and Dr. Paul Weinbren, Nuclear Laboratory, Puerto Rico.

A large number of acute and convalescent sera have shown significant rise in titer in HI and complement tests for the Group B arbovirus dengue I, II, III, and IV, St. Louis encephalitis, Ilheus, and Murray Valley encephalitis viruses.

The major objective has been to isolate the etiological agent responsible for this dengue-like illness. Suckling mice, hamsters, tissue cultures, embryonating eggs have been used

in an attempt to isolate virus from acute sera, mosquitoes, and sentinel mice. Many technical methods such as dilution or concentration of sera, use of carbon particles, and cortisone have been used. Following discussion with Dr. Paul Weinbren in Puerto Rico, radiation of suckling mice is now being tried in an attempt to produce greater susceptibility of the mice for the agent with more rapid illness and death. In many of the mouse groups injected, there has been illness followed by recovery. Passages have been often made with failure to produce illness seen in earlier passages.

A possible etiological agent has been found by the Trinidad Regional Virus Laboratory in three sera sent there from our Jamaican acute serum taken from patients with the dengue-like illness.

Dengue-like Illness in Antigua, West Indies:

Since December there has been an outbreak of dengue-like illness in Antigua, B.W.I. This outbreak is being investigated by Dr. Leslie Spence and his staff at the Trinidad Regional Virus Laboratory.

Unidentified Agents:

Unidentified agents isolated during the past two years have been as follows:

a) Twelve unidentified agents have been isolated from the following specimens of mosquitoes: *Culex nigripalpus*, *Aedes scapularis*, *Aedes tortilis*, *Aedes taeniorhynchus*, *Anopheles grabhami*, *Psorophora confinnis*, *Psorophora pygmaea*, *Mansonia titillans*.

b) Two agents have been isolated from birds - *Mimus polyglottos*, *Columbigallina passerina*.

c) Five agents from chickens - *Gallus domesticus*.

d) One agent from sentinel mice.

It is hoped that the WHO Regional Arbovirus Reference Laboratory at the CDC will be able to assist in the identification of these agents, as the technical staff of our laboratory has been more than fully occupied with material from the EEE epidemic, the dengue-like epidemic, and routine clinical work and teaching.

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

Virus Activity in BBF:

Virus activity in Bush Bush Forest in 1963 was of a high level but of a slightly different pattern than in previous years. In 1963 the dry season was much less dry than usual and consequently the Nariva swamp did not dry completely. Densities of the essential Culex sp. #9 mosquitoes were consequently much higher in the first six months of 1963 than in the comparable period of 1962. These conditions resulted in a burst of virus activity from May through August while in previous years this burst occurred during the last five months of the year.

Forest floor rodent populations which had been declining since the last quarter of 1962 reached a point of virtual extinction during the third quarter of 1963. This, together with the early virus activity resulted in a fourth quarter practically without virus isolations while Culex sp. #9 densities remained high. The whole picture seems to be one caused by the exhaustion of the essential hosts, indicating that alternative hosts may not be readily available in Bush Bush.

Group C Viruses in Trinidad:

Prior to 1963 only two Group C virus types had been recovered from Trinidad. One of these, TRVL 18462 type, was recovered from mosquitoes on one occasion only in 1957 and has not been isolated since. The other type, TRVL 34053-1, a Caraparu-like agent, was first recovered from sentinel mice in 1959 and has been recovered repeatedly since then from mosquitoes, rodents, and sentinel mice.

In 1963 two additional Group C types appeared. These types were studied at this laboratory and at the Belem Virus Laboratory. Results to date are as follows. TRVL 47827, isolated from a pool of Culex sp. #9 on 9 January 1963, appears to be very closely related to the Belem strain of Oriboca virus. TRVL 51144, isolated from a pool of Culex sp. #9 on 23 May 1963, is related to the Belem strains of Marituba and Murutucu but can be distinguished from these two viruses.

Eastern equine encephalitis infection in a Trinidadian horse:

On 22 August 1963, Dr. L. Butcher and Dr. O. Gonzalez, Veterinarians of the Ministry of Agriculture, informed us of a case of equine encephalitis at Las Hermanas Estate, San Raphael, near Arima, in central Trinidad. Blood specimens were collected from the animal on days 1, 2, 3, and 4 of illness. The animal died on day 4 and parts of the brain were collected and tested for virus in suckling mice with negative results. Complement fixation (CF), hemagglutination-inhibition (HI) and neutralization (N) tests carried out on the four serum specimens from the horse with EEE, WEE, and VEE viruses gave the following results:

TRVL No.	Day of Illness	CF			HI			N
		EEE	WEE	VEE	EEE	WEE	VEE	EEE
52700-1	1	Neg	Neg	Neg	1280	Neg	40	Pos
-2	2	Neg	Neg	Neg	20480	40	80	Pos
-3	3	4*	Neg	Neg	40960	40	160	Pos
-4	4	32	Neg	Neg	81920	80	320	Pos

*Reciprocal of serum titer.

These results show clearly that the horse was infected with EEE virus. Failure to isolate virus from the brain was probably due to the fact that the horse died 4 days after onset of encephalitis. EEE virus is usually present in the brain up to the second or third day.

Six other horses on the estate which were in good health were also bled. These animals showed no evidence of infection with any of the three viruses used in the test on the fatal case. A few weeks later one of these animals died but no evidence was obtained that this animal died as a result of infection with an arbovirus.

This is the first laboratory proven equine death from EEE virus in Trinidad. The 1943 outbreak in equines in Trinidad was due to Venezuelan equine encephalitis virus. EEE virus had been previously isolated in Trinidad but only from mosquitoes.

An entomological investigation was instituted at the Las Hermanas Estate on 23 August, the day after the horse fell ill. Over 5,000 arthropods (including 5,035 mosquitoes) were collected and tested for virus. Two viruses were isolated, one from a pool of *Culex virgultus* and one from a pool of

Trichoprosopon digitatum. These viruses are not EEE strains. Ornithological investigations were also instituted in the area. Of 99 birds collected by mist nets between August 28 and September 6, three had neutralizing antibody to EEE virus.

Mosquito surveys:

This laboratory has entered into an agreement with Professor John N. Belkin (University of California) to assist in the study of the mosquitoes of Middle America and adjacent regions, our area of responsibility being the eastern Caribbean. The islands of Grenada and St. Vincent were surveyed in October and November.

Montserrat Serosurvey:

In continuation of our serological survey of humans in the West Indies area, a blood collecting trip was made in September to Montserrat, one of the Leeward Islands of the Lesser Antilles. A total of 214 blood specimens were taken from individuals aged 1-67 years. Of these, 44 (from adults) were tested in the HI test and found to be negative to the following antigens:

Group A: VEE, WEE, EEE, Mayaro, Una.

Group C: Caraparu (TRVL 34053-1), TRVL 47827, TRVL 51144.

Group Simbu: Manzanilla.

Group Bunyamwera: Cache Valley

The 214 sera were studied in the HI test with the following Group B antigens: Ilheus, yellow fever, dengue (TRVL 1751 type) and St. Louis. In addition, several mouse neutralization tests have been completed with these viruses. The results indicate that dengue has been active in the past, but not in recent times.

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

VEE Outbreaks: The incidence of VEE in animals and men has decreased since the last report of August 1963. However, a few cases and the presence of antibodies were reported from all over northern Venezuela, right to the far east.

The following localities were visited: Higuerote district (Edo. Miranda, on the coast east of Caracas), September 1963; Cumana district (Edo. Sucre, 350 Km. east of Caracas), September 1963; Guajira district (Edo. Zulia, where the epidemic started in October 1962), January 1964; Perija district (Edo. Zulia, 100 Km. south of Guajira), January 1964. A large number of mosquitoes were caught and serum samples taken from human patients and many domestic and some wild animals. All these samples are now being processed. As a preliminary result, it is of interest that VEE antibodies were found in several goats, opossums, and donkeys, whereas chicken and turkeys were negative. One assistant (Briceno) contracted a mild form of VEE 24 hours after acting as bait for mosquitoes in the Cumana district. VEE was isolated from the corresponding lot of mosquitoes consisting of Aedes scapularis and from the patient's serum. Identification was performed by neutralization test. This appears to be the first report of VEE isolation from Aedes scapularis.

The results of all investigations of the VEE outbreak from October 1962 until January 1964 are being prepared for publication.

Microtiter equipment was obtained and the HI test performed on a large number of sera. The microtiter technique was found to be very accurate and most useful because of great saving in labor, time, pipets, and sample volume (Sellers, Suarez, Morales, Bergold).

The experiments to find a suitable overlay medium for formation of plaques of certain arboviruses were continued. By modifying the overlay medium of Miles, plaques are formed by all the viruses listed in the accompanying table. The viruses were obtained by the kind and generous cooperation of Dr. Gast-Galvis of the Instituto Nacional de Salud, Bogota; from Dr. L. Spence of the Trinidad Regional Virus Laboratory; and Dr. R. Shope, Belem Virus Laboratory. The use of German agar purified especially for immuno-electrophoresis (pure agar Behring Werke Marburg) was a decisive step in obtaining plaques, particularly of the B Group and Caraparu (Trinidad) and some other viruses. Stimulated by literature reports, the effect of 3-Methylcholantren, Hydrocortisone ("Cortisol", Ciba), Methandrostenolone ("Dianabol", Ciba) and Actinomycin D (Lyovac "Cosmogen" Meractinomycin, Merk Sharp and Dohme) on BHK 21 cells and the formation of plaques of several arboviruses was investigated. BHK 21 cells do not tolerate Actinomycin D (0.06 µg/ml) but the survival of the cell sheets

is greatly prolonged by the steroids, particularly by "Cortisol". this influences very favorably the appearance and size of plaques of slow arboviruses. This effect as well as the influence of the steroids on the production of interferon and virus multiplication is further investigated.

Dr. R.F. Sellers has been appointed Deputy Director of the Research Institute (Animal Virus Diseases) in Pribright, England, and will leave shortly; thus, there will be a vacancy here for an experienced veterinary virologist (Bergold).

VIRUSES PRODUCING PLAQUES IN BHK 21 CELLS

VIRUS	SOURCE	APPEARANCE OF PLAQUES	DAYS P.I.	AGAR	NOTES
Group A					
Una	G	very clear	3	Noble	
Group B					
FN Yellow fever	G	very clear	4	Noble	German better
TRVL " "	Sp	very clear	5	German	+ Cortisol better
IVIC 1 " "		readable	6	German	
IVIC 2 " "		very clear	5	German	
JSS " "	G	readable	5	German	
17D " "	P	very clear	5	German	+ Cortisol better
17D " "	G	clear	5	German	
Bussuquara	G	very clear	5	Noble	
Group C					
Caraparu Belem	Sh	indistinct	6	German	
Caraparu Trinidad	Sp	readable	6	German	+ Cortisol better
Bunyamwera Group					
Guaroa	G	very clear	3	Noble	
Wyeomyia	G	clear	4	Noble	
Cache Valley	Sp	very clear	3	German**	
Kairi	Sp	very clear	4	German**	
Guama Group					
Guama	Sp	clear	5	German**	
Bimiti	Sp	very clear	5	German**	
California Complex					
Melao	Sp	clear	6	German**	
Simbu Group					
Oropouche	Sp	clear	7	German**	
Manzanilla	Sp	very clear	6	German**	
Triniti Group					
Triniti	Sp	very clear	4	German**	
VSV Group					
New Jersey	-	very clear	1-2	Noble	
Indiana	-	very clear	1-2	Noble	
Cocal	Sp	very clear	1-2	Noble	
Other Groups					
Anopheles A	G	very clear	3	Noble, German	
Anopheles B	G	readable	6	German	

*G=Gast-Galvis; Sp=Spence; P=Porterfield; Sh=Shope.
**Noble not checked.

REPORT FROM DR. H.A.E. VAN TONGEREN
DEPARTMENT OF MEDICAL MICROBIOLOGY, VRIJE UNIVERSITEIT
AMSTERDAM, NETHERLANDS

A Serological Survey of Surinam Against Arboviruses Group A (preliminary study).

As a dam of something over 2 km in length and a hydroelectric generation station is being built in the Surinam River, just above Affobakka, which will probably be completed by 1965, sera were collected, mainly from adults from 15 years and older, in 2 areas, the north and the south part of the future Brokopondo lake, which will be formed.

This lake is to cover an area of about 1.500 square km. in the interior of Surinam. As this future lake may vary the ecologic situation in this area, this suggested the idea to perform a serological survey by means of neutralization tests on the incidence of some 20 arboviruses known to occur in countries surrounding Surinam.

This preliminary report gives the result of the 75 sera collected in the northern area against arboviruses belonging to group A.

Chikungunya - Mayaro - Semliki Forest complex.

Twenty-four sera (32%) were negative for Mayaro virus. Twenty-nine sera (38.7%) for Semliki Forest virus and 35 sera (46.7%) for Chikungunya virus. Twenty-four sera were triple negative for all 3 viruses, one Mayaro positive serum was negative for Semliki Forest, 4 were negative for both. Semliki Forest and Chikungunya and 7 others were negative for Chikungunya only. All neutralizing indices against Mayaro virus were higher as compared with those against Semliki Forest virus and again the latter were higher as compared with the neutralizing indices against Chikungunya virus. When interpreting these serological results, the view is expressed that in the interior part of Surinam infections in humans are due to infection with Mayaro virus rather than Semliki Forest and Chikungunya virus.

WEE - Sindbis viruses.

Only 1 doubtful positive serum was found against WEE virus. One other serum repeatedly showed a log neutralization index of 3.45 against Sindbis virus. This serum was negative for WEE antibody but positive for VEE and EEE antibodies.

EEE Virus.

Eleven serum samples (5 females and 6 males) showed neutralizing antibody against EEE virus, 8 of these contained antibody against both VEE and EEE viruses; 3 (2 females and 1 male) were positive for EEE (log NI = 1.75) and negative for VEE; 4 (2 females and 2 males) showed a higher NI against VEE than against EEE and 4 (1 female and 3 males) showed a higher NI against EEE than against VEE.

The log NI against EEE varied between 1.75 (5 x) tot 4.85 (5 x).

VEE virus.

Twenty three sera were positive for VEE antibody (8 females and 15 males). The log NI indices were >2.25. The donors were aged 17 to 65 years.

REPORT FROM DR. S.R. PATTYN
BACTERIOLOGY DEPARTMENT, INSTITUTE FOR TROPICAL MEDICINE
ANTWERP, BELGIUM

Our studies on the production of infectious virus and viral hemagglutinins in tissue cultures of chick embryos and HeLa cells in the presence of different media, were continued.

A preliminary report on some of the results is in preparation, and we hope to extend our observations so as to be able to present a more complete picture for the next issue of the Info-exchange.

A study was also started on the possibility of latent infection of some animals by arboviruses, in trying to reisolate the virus from tissue cultures of organs from animals at various time lapses after artificial infection. The study has not sufficiently progressed to present results.

Capture of local mosquitoes was started and several batches were already inoculated in tissue cultures of HeLa cells, chick embryos, human amnion, and in baby mice. The results are negative up till now.

Our serological work was done in collaboration with Drs. Bres and Chambon of the Institut Pasteur de Dakar. We tested in the plaque-reduction test as described by Porterfield sera from Senegal. Here also the results are incomplete, but many sera have antibodies for Chikungunya virus or the related O'Nyong-nyong virus.

We did also "mixed hemadsorption" tests. The system works well with vaccinia virus but up till now we did not succeed in applying this technique to Chikungunya or West Nile viruses.

The laboratory section on fluorescent antibody staining was also started and we hope to be able to present results from this field in the future.

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

Three hundred sixty-eight sera were tested by the HI test in an attempt to find antibodies to viruses of the tickborne complex. The technic used was that of Clarke and Casals. The antigen was brains of suckling mice infected with the Graz strain of Central European tickborne encephalitis virus.* These brains were treated by protamine in borate buffered saline pH 9.3. The virus was sedimented by centrifugation at 105,000 g. The pellet was resuspended in the same buffer. Through the tests we used 6 H.A. units of antigen. The red cells were goose cells. The final pH of the reaction was 6.8. All the sera tested were kaolin treated and goose cells adsorbed.

RESULTS OF THE HI TESTS

Origin of Sera	Number of Sera		
	Tested	Positives	Negatives
Children with neurological symptoms	27	0	27
Serological survey	341	1**	340

**HI titer of the positive serum: 1/40

The only serum that showed a positive reaction in the HI test came from a woman of the village of St. Vith. She was 52 years old and had no history of previous illness or vaccination or of travelling in far-away countries. In two different neutralization tests we could not find a significant protection of the mice, although the survival time was slightly increased.

Our survey was biased by the fact that the sera, otherwise randomly taken, came without exception from people in excellent health which were accepted as blood donors***; as a consequence the age and sex distribution was that of the blood donors. Our survey therefore gives almost no information on the younger and older age groups, and less information on females than males. The sera were collected in six villages located in the eastern part of the country, near the German border.

Age, Sex, and Geographical Distribution

Age Groups	11-20		21-30		31-40		41-50		51-60		61-70		Totals		
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Both
Villages:															
Vielsalm	0	3	3	1	5	0	1	5	2	4	0	1	11	14	25
Ferriere	0	0	4	4	6	0	1	2	2	4	1	0	14	10	24
Eupen	2	0	27	4	12	7	11	18	7	12	0	0	68	40	108
Welkenradt	0	0	12	2	11	3	8	8	6	9	3	5	40	27	67
St. Vith	1	0	16	5	15	1	8	2	5	2	1	1	46	11	57
Stavelot	0	0	10	0	13	5	15	0	8	6	2	0	48	12	60
Totals	3	3	72	16	71	16	44	35	30	37	7	7	227	114	341
	6		88		88		79		67		14		341		

Additional surveys are planned with more sera and more antigens.

De Mayer (personal communication) has observed that the number of plaques appearing under agar, when the viruses are allowed to adsorb on the cells for various lengths of time, is lower when the agar overlay is poured immediately after the washing out of the unadsorbed virus than when the agar overlay is poured one hour later. He works among others with Sindbis and vaccinia and uses Difco purified agar.

We noticed that this holds true with Semliki Forest virus and vaccinia under an overlay made of Difco Noble Agar. If the overlay is poured immediately after the washing out of the unadsorbed virus, the number of plaques increases as a direct function of the adsorption time. If the overlay is poured one hour later, the number of plaques increases as a function of the square root of the adsorption time as it should (cf. Allison and Valentine, Biochem. Biophys. Acta, 1960, XL, 400). This is an argument in favor of the hypothesis that agar can exert an inhibitory effect on the process of infection between

the stage of simple adsorption of the viral particle on the cell surface and the irreversible attachment of the virus to the cell.

*This strain was kindly supplied by Prof. van Tongeren.

***The sera were kindly supplied by "Le Service des Transfusions et la Croix Rouge".

REPORT FROM THE DEPARTMENT OF VIROLOGY UNIVERSITY OF HELSINKI, FINLAND

At the Symposium on Biology of the Tick-Borne Encephalitis Complex in Bratislava in 1960, we reported the isolation from Ixodes ricinus ticks of some mouse pathogenic agents other than typical tick-borne encephalitis viruses. At the moment, five such agents (Table 1) have been studied further by our arbovirus group (N. Oker-Blom, M. Brummer-Korvenkontio, L. Kaariainen, A. Salminen, P. Weckstrom).

The agents caused paralysis in suckling mice inoculated intracerebrally with mean titers of 10^{-7} per ml. Usually no symptoms in adult mice inoculated intracerebrally were seen. Occasionally, however, an adult mouse showed symptoms of paralysis. The main incubation period in infant mice was six days. Re-isolation was successful with four (S 23, S 27, A 21, A 101) out of these five agents, suggesting that they do originate from nature. Strain S 23 could be passed through a Seitz filter and was ether and desoxycholate sensitive.

Cross neutralization tests in mice show that at least two of the agents (S 23 and A 21) in this group are antigenically related. No neutralization was obtained by a tickborne encephalitis immune serum and serum prepared against one of the agents of this group did not neutralize a tickborne encephalitis virus. It may be mentioned that no neutralization of the representative agent of this group was obtained with a lymphocytic choriomeningitis immune serum.

Table 1

Origin and Properties of Some Viruses Other than Tick-Borne Encephalitis Viruses Isolated from Ixodes ricinus Ticks in Finland in 1959-1960

Strain	Collection of Ticks		Number of Ticks in Pool
	Date	Locality	
S 23	26.2.60	Uukuniemi, S.E. [1]	182 ♀ engorged
S 27	27.6.60	Uukuniemi, S.E.	6 ♀ 20 N
A 21	12.6.59	Jomala, S.W. [2]	2 ♀ 22 N 49 L
A 101	25.8.59	Kumlinge, S.W.	2 ♀ 41 N
A 17	12.6.59	Finström, S.W.	11 ♀ engorged

[1] S.E. = South East Finland

[2] S.W. = South West Finland (Aland islands)

Table 2

Results of Complement Fixation Tests with Tissue Culture Antigen of Strain Uukuniemi S 23 and Different Arbo Virus Immune Sera

Immune Serum	CF Titre with S 23 TC Antigen
Group A	
Western Equine Encephalitis, Guinea Pig	< 10
Eastern " " , " "	< 10
Group B	
Tick-Borne Encephalitis BMF, Guinea Pig	< 10
Japanese B, " "	< 10
Yellow Fever, " "	< 10
St. Louis Encephalitis, " "	< 10
Others	
Tahyna, Mouse	< 10
Calovo, "	< 10
Colorado Tick Fever, Mouse	< 10
Uukuniemi S 23, Guinea Pig	160

The representative strain for which we now suggest the name Uukuniemi S 23 induces cytopathic changes in cultures of a continuous line of monkey kidney cells, BSC cells. In such cultures, no hemagglutinins have so far been obtained but a complement fixing antigen was produced reacting with the homologous serum as well as with the immune sera prepared against all the other agents in this group, supporting the assumption that these five agents are antigenically related.

Further attempts to classify the agents in question were done by testing a number of arbovirus immune sera for complement fixing antibodies to a tissue culture antigen of the representative strain (Table 2). None of the available immune sera reacted with antigen of the strain S 23 in the complement fixation test.

With the available methods, it has thus not been possible to classify the agents of this group but at least for the present it seems justified to place them in the group of arboviruses.

It is thus evident that viruses other than typical tickborne encephalitis viruses occur in ticks in Finland. Further studies will reveal to what extent they are related to some viruses recently isolated from ticks for instance in Great Britain, USSR, and Czechoslovakia, or some other known arboviruses.

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

New Isolated Virus from Ticks Ixodes ricinus and Small Rodents in Czechoslovakia:

In April 1963, an expedition was carried out in natural focus of tickborne encephalitis in the environment of the town Zlate Moravce (Central Slovakia). The studies were performed on western part of Tribec Mountain. This area is inhabited by a high density of hunting game, small rodents, and insectivores. On April 18, collection of ticks Ixodes ricinus from vegetation was initiated. The ticks collected in the field were tested in separate samples, according to the stage of ticks. Each sample was inoculated into tube cultures of chick embryo cells and in suckling mice.

From 3719 ticks, 9 cytopathic agents were isolated (Gresikova et al., 1964). Cytopathogenic effect of "Tribec" virus became apparent in 2 days after inoculation of cultures. The virus forms plaques under agar and the agent causes fatal infection in suckling mice. The virus is pathogenic for chick embryos but not pathogenic for other animals (adult mice, guinea pigs, monkeys).

This report records the first isolation of "Tribec" virus from different stages of ticks Ixodes ricinus. The "Tribec" virus was isolated from adult ticks (♀♀ and ♂♂) and nymphs; one strain was isolated from the tick engorged on white mice (Table 1). The estimated incidence of virus-carrying ticks in various seasons ranged from 0.1 per cent to 0.3 per cent. During spring season, the highest incidence of infected ticks was observed.

Specimens of bank vole (Clethrionomys glareolus) and pine vole (Pitymys subterraneus) animal hosts of Ixodes ricinus were captured in traps. From living animals blood samples were taken by cardiac puncture and tested for virus and neutralizing antibodies. The virus was isolated and reisolated from the blood of two animals: Clethrionomys glareolus and Pitymys subterraneus, in first passage in tissue cultures of chick embryo cells (Table 2). The viruses were subjected to identification procedure. Complement fixation test confirmed that two agents from Pitymys and Clethrionomys are identical with the "Tribec" virus.

"Tribec" virus is not related to tickborne encephalitis virus and to mosquito-borne viruses as western and eastern equine encephalitis viruses, St. Louis, Japanese B encephalitis virus (Table 3). In complement fixation test, the "Tribec" virus showed antigenic relationship to Kemerovo virus (Chumakov, 1963), but the viruses are distinct from each other (Gresikova et al., 1964). "Tribec" virus is identical with cytopathic agents isolated from the ticks Ixodes ricinus collected near Bratislava (Libikova et al., 1964).

References:

Chumakov et al., 1963. Report on the isolation from Ixodes persulcatus ticks and from patients in Western Siberia of a virus differing from the agent of tick-borne encephalitis. Acta virol. 7, 82-83.

Gresikova et al. 1964. Ecology of Tribec virus. Acta virol. In press.

Libikova et al. 1964. Cytopathic viruses isolated from Ixodes ricinus ticks in Czechoslovakia. (Letter, Acta virol. In press).

Table 1

Isolation of Tribeč Virus

from Different Stage of Ticks Ixodes ricinus

Ticks		Positive isolation experiments
Stage	Number	
Females	615	2
Males	588	3
Nymphs	2 028	1
Engorged females	177	2
Engorged nymphs	311	1

Table 2

Host Range of Tribec Virus

Small mammals	Isolation experiments			
	No	July	No	October
Apodemus flavicollis	1	-	17	-
Clethrionomys glareolus	1	1 +		
Micromys minutus			1	-
Mus musculus			5	-
Pitimys subterraneus	7	1 +		
Talpa europea	6	-	2	-
Erinaceus roumanicus	2	-	8	-

No = number

+ = positive isolation experiments

- = negative isolation experiments

Neutralization Tests with Tribec Virus

Antiserum		Neutralization Index
Group A	Western Equine Encephalomyelitis	0
	Eastern Equine Encephalomyelitis	0
Group B	Tick - borne Encephalitis	0
	St. Louis Encephalitis	0
	Japanese B Encephalitis	0
Ungrouped	Kemerovo	1000

REPORT FROM DRS. J.S. PORTERFIELD AND J.P.T. BOORMAN NATIONAL INSTITUTE FOR MEDICAL RESEARCH, LONDON, ENGLAND

The original AR 749 strain of Middelburg virus has been found to be composed of a mixture of particles giving rise to large and small plaques in monolayer cultures of chick embryo fibroblasts incubated under agar. The two variants breed true and are antigenically similar. A further strain of Middelburg virus, AR 2196, received from Dr. B.M. McIntosh at the South African Institute for Medical Research, Johannesburg, has been found to contain only particles which give rise to large plaques.

Recent virus isolation studies in the Lagos area of Nigeria, carried out in collaboration with Dr. C.C. Draper, have resulted in the isolation of 8 strains of virus from a little over 100,000 mosquitoes. One of these strains was from a mixed pool of Culex species, one from Anopheles species, one from a mixed pool of Aedes species and the other five from Mansonia africana and Mansonia uniformis. Two of the strains appear to be similar to Chikungunya, two to Bwamba, and one to the "Ukauwa" strain of Bunyamwera; the remainder have yet to be identified. Antibodies to these identified strains have been found to be widespread in human and animal sera from the area.

The transmission of Ilesha and Ukauwa viruses by Aedes aegypti has been accomplished; both this species and Mansonia africana appear to be capable of acting as efficient vectors of Ukauwa virus.

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

I. Studies in 1962-63 of louping ill in Ayrshire (SW Scotland).

A. Small mammals. One hundred thirty-one were trapped in 1963 and louping ill virus has now (1961-63) been isolated from 2 of 30 (7%) of Apodemus sylvaticus from infected farms and from 1 of 99 (1%) of Sorex araneus. Antibody to louping ill virus has been found in 10% of A. sylvaticus and M. agrestis and in 25% of S. araneus. That small mammals become infected is therefore proved, but evaluation of their importance requires further study of their tick infestation rates and of whether they circulate virus in high enough concentration to infect ticks.

B. Tick collections from vegetation. Drags were made daily from March 19 to April 26 and weekly or fortnightly from May to October. The nymph population in 1963 was markedly higher than in 1962. A total of 1318 larvae, 1976 nymphs, and 40 adults were tested in pools for virus. Two isolations were made from nymphs from one area, giving an infection rate of 1:610. Three areas of defined botanical composition were compared and a clear correlation found between tick population and percentage cover by the soft rush (*Juncus effusus*). The numbers collected could be correlated with maximum air temperature.

C. Tick population dynamics. There appears to be a 3-year cycle of stage dominance (larva-nymph-adult-larva) due either to a vole year (e.g. 1959) or to the severity of a particular winter. Evidence suggests that years of exceptionally high nymph populations (e.g. probably 1960) are the years when high lamb losses can be expected provided that they have been preceded by a few years of low infection rates so that many lambs lack maternal antibody.

D. Fenced yearling sheep. Five yearling sheep were fenced on a 2-acre plot and examined weekly for ticks, viremia and antibody from April to October. One hogg had pre-existing antibody but no evidence of infection of these animals was found during the period. Twenty-six larvae, 233 nymphs, and 239 adults were tested for virus without success.

E. Other work on sheep.

1. Serology in experimentally infected sheep. Studies in collaboration with Mr. O'Reilly of experimentally infected sheep have shown that while neutralizing antibody is very long-lasting, HI antibody is lost fairly quickly. In 50% of animals, titers had fallen below the detectable level 7 months after infection. By about 9 months, 75% had lost detectable HI antibody but the 25% remaining seem to have long-persisting low titers. Those with the highest titers of HI antibody initially tend to remain positive longest. This fits very well with field observations and indicates that HI antibody, at any rate in young animals, is usually evidence of infection during the current season. It remains to be determined whether repeated infections cause boosts in HI antibody and/or longer persistence.

2. Surveillance of infection rates on the study farms. With the collaboration of Mr. Wilson and his colleagues and with Mr. O'Reilly, blood samples are being examined serologically

each year from yearling sheep. Blood samples are obtained in March, June, and September. The infection rates appear to have been falling steadily over the last two years: on Camlarg Farm from about 80% in 1960 and 1961, to 25% in 1962 and to 15-20% in 1963. Dalcairnie Farm has shown a similar fall while Knockgray Farm has remained virtually free of infections.

3. Studies in 1961. A paper entitled "The epidemiology of louping ill in Ayrshire: the first year of studies in sheep", will appear in the March number of the Journal of Hygiene.

II. Studies of encephalitis in Sarawak, North Borneo.

The three strains of virus isolated from mosquitoes in December 1962/January 1963 have now been provisionally identified: MS117 and MS128, isolated from pools of *Culex tritaeniorhynchus*, appear to be closely related to or identical with Tembusu (AMM1775). MS50, isolated from *Aedes* (*Cancraedes*) *curtipes*, is a member of the Bunyamwera group. Cross gel-precipitation and absorption studies are still in progress. It appears to be more closely related to the prototype Bunyamwera than to other strains tested including Batai (AMM2222) the Malayan member of the group.

REPORT FROM THE EAST AFRICAN VIRUS RESEARCH INSTITUTE ENTEBBE, UGANDA

Studies on bats were started again last June and have already given a number of interesting findings:

1. HI antibody studies. Acetone-extracted sera from 168 bats have been screened against a range of arboviruses by HI with the following proportions of positives:

18/22 fruit bats, *Eidolon* and *Epomophurus* spp.
90/97 roof dwelling *Tadarida* spp.
5/49 Cave dwelling bats *Hipposiderus*, *Miniopterus*, *Nycteris*, *Rhinolophus* spp.

Virtually all the positives were with viruses belonging to Casals' Group B. The titers were usually highest with Entebbe Bat Salivary Gland virus but many were also positive with yellow fever, Zika, H 336, and West Nile antigens. The two exceptions were a serum from *Hipposiderus caffir centralis* K.

Anderson which showed Chikungunya HI antibodies to a titer of 1/40 and a serum from Tadarida (Mops) condylura (A. Smith) which gave a positive reaction at 1/20 with Bunyamwera antigen.

2. Isolation studies. In view of the very high incidence of Group B antibodies pooling of bat material for isolation studies was soon stopped. Material taken for isolation studies has comprised salivary glands and on occasion brain, blood, and spleen.

Approximately 300 bats have been processed, 125 of these have been T.(M.) condylura and 6 strains of virus have been isolated from their salivary glands. Isolation work with other species has been unproductive.

HI screening of the 6 new isolates shows that they belong to Casals' Group B and are apparently different from the viruses of this Group registered as occurring in Africa. There appear to be at least two different viruses and one shows a close relationship to a strain of virus (DAKAR 249) which was isolated from the salivary glands of Scotophilis spp. by Dr. Bres in West Africa.

3. Viremia studies. The technical problems of maintaining bats alive have limited this work. It has however, been found that Bunyamwera virus circulates for a period of not less than 7 days with titers of at least 3 log LD_{50} in Tadarida (Chaerephon) pumila (Cretzschmar), without obvious signs of illness.

Using early passage local yellow fever virus in a number of T.(M.) condylura, no circulation was obtained over a period of 21 days. It is to be remembered that this species has shown a very high incidence of Group B antibodies. At the time this experiment was carried out it was not known to us that Sulkin had been successful in yellow fever virus circulation experiments with bats of the same genus. Our failure may be related to a strain difference as he suggests.

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

Investigations on virus reservoirs were initiated with the study of bats. Captures had begun by July 1962 in different urban centers in Senegal and were carried on during 1963.

Bats are caught under roofs of buildings and sometimes in cellars or in wells. They are only insectivorous species,

and Pr Aellen from the Museum d'Histoire Naturelle in Geneva, identified among them: Scotophilus nigrita nigrita (Schreber), Taphozous sudani sudani (Thomas), Tadarida condylura wonderi (Sanborn) Other species are awaiting identification.

Bats were generally processed in pools of 20 to 30 animals, sometimes more. Salivary glands were harvested, and, after grinding, roughly a 10% suspension is prepared. After spinning at 4000 rpm, suckling mice, one day old, are inoculated with suspensions, by IC and IP. The number of pools inoculated according to the centers of successful isolations are as follows:

Center	Bats	Pools	Strains
1962:			
Thies	65	2	0
Pire	120	5	1 DAK/249
Kelle	21	1	0
M'Backe	19	1	0
Gossas	76	2	0
Gagnick	35	1	1 CHIK
Bile	45	1	0
Rao	53	3	1 CHIK
1963:			
Sakal	20	1	1 n.i.
M'Pal	50	2	0
Macka	50	2	0
Koupentoum	43	1	0
Birkelane	43	2	2 DAK/249
Koungheul	113	5	5 DAK/249
Mekhe	350	13	1 DAK/249
"	"	"	3 n.i.
Pire	100	4	1 n.i.
Boulal	234	10	2 n.i.
Linguere	4	1	0
Kelle	253	11	4 n.i.
Mouk Mouk	20	1	1 n.i.
Kebemer	29	1	0
N'Dande	4	1	0
Dala	196	11	2 n.i.
N'Doula	40	2	0
M'Backe	384	19	11 n.i.
Touba	160	8	2 n.i.
TOTAL	2527	111	38

(n.i. = not identified)

It may be seen that bats were found carrying arboviruses in 14 of 26 centers investigated. Thirty-eight strains of viruses killing newborn mice with paralysis were isolated from 111 pools prepared with 2527 bats. The frequency of isolations, as judged by one and a half years of observation, does not depend on a seasonal factor; bats act as reservoirs all year long. It was also proved that a virus may persist among the bats of a same roof, for several weeks, as was seen at Koungheul.

Date	No. bats	Pools	Strains
25/7/63	23	1	1
"	22	1	1
7/8/63	24	1	1
14/8/63	21	1	1
"	23	1	1

The identification of all 38 strains is not yet completed, but provisional data are available for 11 of them. It was confirmed by E.A.V.R.I. (Dr. Haddow and Dr. Williams) that the strains from Gagnick and Rao were CHIK. The strain DAKAR/249 is perhaps a new one in Group B. This last strain appears to be frequent in Senegal, as it was also isolated in 2 pools at Birkelane, 5 pools at Koungheul, and at least 1 pool at Mekhe. Some features in the mice dying during passage allowed us to suppose several other samples of DAK/249 belong to the 27 unidentified strains.

The pools of about five sera from some of these bats were surveyed by HI after they were treated by acetone and goose red cells according to the Clarke and Casals (1958) method. The antigens used were CHIK, O'NN, DAK/249, YF, ZIKA, UGS, WN, BUN. Thirteen from 34 pooled sera exhibit antibodies only against DAK/249 at dilutions varying from 1/20 to 1/1280. In two cases heterologous reactions with other antigens from group B appeared when DAK/249 antibodies occurred at high level. There were no antibodies for CHIK, O'NN, or BUN. It is noticeable that almost all pooled sera coming from the same center were positive while they were all negative in another center. It looks as if all members of one colony had been infected while all members of another colony were uncontaminated. In two cases a strain of viruses was isolated in animals whose sera were carrying DAK/249 antibodies. It will be interesting to see if identification of these strains is also DAK/249.

All these data clearly point out the very important role played by bats as reservoirs for arboviruses.

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

Systematic studies on the haemaphysalid fauna in and near the area infected by Kyasanur Forest disease have been completed (in conjunction with Dr. Harold Trapido). New species names have been made available and status of old haemaphysalid species names has been established and clarified so that Virus Research Center personnel can at last begin publication of faunal keys and reports on biological and transmission studies in which haemaphysalid species were included.

With completion of this phase, similar work has commenced on the haemaphysalid fauna elsewhere in southern Asia. Material contributing to this study is welcomed.

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

Phlebotomus fever. Identifications of viruses isolated from field-collected *Phlebotomus papatasi* have been completed. To date, 21 strains of phlebotomus fever virus have been recovered from approximately 119,000 sandflies collected in the environs of Cairo. Nineteen of the strains were Sicilian type and two were Naples type. In a comparison of isolation methods, non-engorged females proved a better source of virus than engorged females. Similarly, females held in the laboratory to allow complete blood meal digestion yielded more isolates than those processed immediately after collection. Interestingly, 2 strains of virus were recovered from pools of male sandflies which are not known to feed on vertebrates. The source of virus for males is being investigated.

Studies on virus multiplication in the sandfly revealed that the multiplication rate and ultimate concentration are dependent on the ambient holding temperature. Infection rates also appear to be temperature dependent.

Transovarial passage of Sicilian type virus has been demonstrated in a small percentage of sandflies. The virus has been detected in male and female first generation progeny of experimentally infected flies.

The search for extra-human hosts continues, but as yet no mammalian or avian species has been found to possess sandfly fever virus antibody.

The following entomologic information on P. papatasi was obtained: a) proof that the presence of blood in the larval diet is not an important factor in autogenic development; b) determination of the feeding periodicity and longevity of sandflies in a quasi-natural environment.

West Nile Fever. Persuant to the recovery of West Nile fever virus from the African bird tick, Argas reflexus hermanni, a laboratory colony of this species has been established preparatory to determining its vector potentialities.

Migratory Bird Work. Studies designed to assess the role of migratory birds as long-distance disseminators of viruses continued. During the spring and fall 1963 migration seasons, the bloods of 1,200 birds were processed for virus isolation, bringing the total processed to date to nearly 4,400. Forty-three virus strains have been recovered from bloods of birds migrating from Europe and Asia to Africa; thirty-nine were recovered from 2 avian families, Sylviidae (warblers) and Turdidae (thrushes and chats). Preliminary immunologic evidence indicates the presence of at least five distinct agents. The isolates are currently being compared with known arbo-, entero-, and myxoviruses.

In addition to the viruses from bird bloods, three viral strains were isolated from Hyalomma and Haemaphysalis ticks parasitizing the birds. These agents seem to be unrelated to any of the bird blood isolates.

Persons actively engaged or otherwise interested in the isolation and identification of viruses from migrating birds are invited to correspond with Dr. Schmidt.

REPORT FROM THE DEPARTMENT OF BACTERIOLOGY AND VIROLOGY AMERICAN UNIVERSITY OF BEIRUT, BEIRUT, LEBANON

The first laboratory studies of arthropod-borne viruses in Lebanon were carried out by our group in 1955. Using commercially prepared antigens from SLE, EEE, and WEE, 121 serum samples obtained from various age groups were studied by the CF test. In 1960, a larger number of serum samples was studied using again commercially prepared antigens from SLE, Jap. B., EEE, and WEE. The results of these preliminary determinations

are given in Table 1. It is apparent from these results that while a small percentage of the individuals tested have shown antibodies to SLE, EEE, and WEE in 1955-56 determinations, these reactions have been absent (except for 2 positive reactions with SLE) in the 1960-61 determinations. The reason for this discrepancy cannot be explained.

Interest in work on arboviruses was revived in 1961, when 60 serum samples obtained from individuals in various age groups were sent to the Rockefeller Foundation Virus Laboratories, New York, where Drs. Theiler and Casals kindly carried out HI tests, using a number of antigens belonging both to group A and group B arboviruses. All sera were negative with the group A antigens (Chikungunya, Sindbis, Semliki) while 15% of sera from individuals in the age group 0-29 and 43% in the age group 30-69 were positive with group B antigens (mostly West Nile and dengue type 1). However, the "positive results with group B, in general, did not give a clear-cut diagnostic pattern". The results of these determinations are given in Table 2.

Following the report of the Rockefeller group, work on seroimmunity of the Lebanese population to group B arboviruses was resumed together with attempts to isolate indigenous viruses from mosquitoes. Using the extraction methods and the technics described by Clarke and Casals a small number of serum samples collected from various districts of Lebanon were tested against West Nile virus. A high percentage of the samples tested gave positive results with this antigen (Table 3). Work is being carried out at present to test similar samples with antigens prepared from dengue, I, II, III, and IV, and yellow fever viruses using the hemagglutination inhibition and neutralization tests.

Mosquitoes, caught in various districts of Lebanon, were tentatively identified in this department as *Aedes mariae*, *Culex molestus*, *C. laticinctus*, *C. hortensis*, *Theobaldia longiareolata*, *T. annulata*, and *T. subocrea*. Isolation studies using various pools of these mosquitoes are also being carried out at present.

Table 1

Sero-immunity to SLE, Jap. B, EEE and WEE Viruses Among Lebanese During the Years 1955-56 and 1960-61 as Determined by the CF Tests

Antigen	Year 1955-56			Year 1960-61		
	Number of Sera Tested	Number of Positives	Per cent Positive	Number of Sera Tested	Number of Positives	Per cent Positive
SLE	121	7	5.8	421	2	0.48
Jap. B	N.D.	N.D.	-	349	0	0
EEE	121	7	5.8	371	0	0
WEE	120	4	3.3	371	0	0

N.D. = Not done

Table 2

Results of Hemagglutination-Inhibition Tests to Arboviruses with Sera from Lebanon

Age Group	Number of Sera	No. of Sera Positive with		Per cent Positive Group B
		Group A[1]	Group B[2]	
0 - 9	10	0	0	0
10 - 19	10	0	2*	20
20 - 29	10	0	1	10
30 - 39	10	0	4*	40
40 - 49	10	0	3*	30
50 - 69	10	0	6	60

[1]Chikungunya, Sindbis, Semliki

[2]West Nile, Meningo-encephalitis of turkeys, dengue type 1, dengue type 2, RSSE, Japanese B, and yellow fever.

*One sample positive with yellow fever (vaccinated or probably vaccinated).

Table 3

Results of Hemagglutination Inhibition Tests to West Nile Virus with Sera Collected from Lebanon

Age Group	Number of Sera Tested	Number Positive	Per cent Positive
0 - 9	15	9	60
10 - 19	14	6	42.8
20 - 29	17	15	88.2
30 and over	38	30	78.8

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

Cross neutralization tests between Japanese B encephalitis (JBE) and dengue viruses:

Immune sera were obtained from adult male rabbits inoculated repeatedly with infected mouse brain homogenates of JBE (G1 strain) and dengue (type 1, Mochizuki strain) viruses, respectively. Neutralization indices (NI) were determined by the method applying Reed-Muench formula using serially diluted virus and constant amount serum. One of the typical examples is as follows:

Immune Serum	NI against virus of: JBE	Dengue
Anti-JBE	≥10,000	0
Anti-dengue	0	≥1,700

It is evident that there is no overlapping reaction between JBE and dengue type 1 viruses, at least through the adopted procedure. Based on this finding, the below-described studies were undertaken.

Reference: Lennette, E.H.,1956, in Diagnostic Procedures for Virus and Rickettsial Diseases, Am. Publ. Hlth. Assn., pp. 1-51.

Detection of anti-dengue neutralizing antibody in the sera of residents of Japan:

Sera were collected during 1962-63 from residents of Kyoto City (without any history of dengue epidemic) and of Nagasaki City (with history of dengue epidemics in 1942-44). (The specimens from Nagasaki were obtained through the kindness of Dr. A. Kondo and Dr. Y. Aoki, of Nagasaki University.) All tested individuals were born prior to the dengue epidemics in Japan (1942-45), the age distribution ranging from 20 to 73. NI's against type 1 Mochizuki strain virus were measured, obtaining the following results.

NI	No. of sera from: Dengue-free area (Kyoto)	Dengue-infected area (Nagasaki)
>32	0	4*
32 - 3	0	0
3 - 0.3	41	34

*Tests were repeated, obtaining practically the same titers.

Significant anti-dengue antibody (NI higher than 32) was demonstrated in 4 out of 38 sera from Nagasaki City, whereas all of 41 specimens from Kyoto City had no antibody.

Among the sera from Kyoto, anti-JBE antibody (NI equal to, or higher than 10) was detected in 25 out of 39 samples (ratio of positiveness: 64%).

References:

Hotta, S., 1953, J. Trop. Med. & Hyg., 56, 83.

Theiler, M., Casals, J., and Moutousses, C., 1960, Proc. Soc. Exp. Biol. & Med., 103, 244.

Production of anti-dengue neutralizing antibody in Japanese monkeys (Macaca fuscata) inoculated with dengue virus of tissue culture origin:

Type 1 Mochizuki strain virus was used. "Live virus" was harvested in fluid medium from infected monolayer cultures of fuscata monkey kidney cells. "Formalinized virus" was prepared by mixing the infected fluid with formalin (35% aqueous solution of formaldehyde) at concentration of 0.2% and holding it at 4°C for 2 weeks; the mouse-infectivity was completely lost by this treatment.

Monkeys employed were 1 to 3 year old males, bred in an experimental station of the Monkey Center of Japan. They were in good physical condition and had no neutralizing antibodies against dengue type 1 (Mochizuki strain) and JBE (G1 strain) viruses. For 6 months prior to, as well as during the present experiments, they were kept in a mosquito-proof room of the Kobe Medical College.

Each monkey was inoculated, two times at an interval of 3 weeks, intracutaneously with 0.1 ml each of either formalinized or live virus. No abnormal signs were apparent in all of them for 6 weeks following inoculations. Sera were collected from each animal and NI's measured.

Monkey		Prior to[b] inoculation	2 weeks after 1st inoculation	1 week after (b) 2nd inoculation
Inoculated with (a)	No.			
Live virus (undiluted)	1	0	10	100
	2	0	5	≥ 100
Live virus (Diluted 100-fold)	3	0	56	≥ 100
	4	0	0	56
Formalinized virus	5	0	0	0
	6	0	0	0

(a) Titers (mouse intracerebral LD_{50}/0.02ml) of the original virus were $10^{4.75}$ for the 1st inoculation, and $10^{5.0}$ for the 2nd inoculation. Formalinized virus was prepared from undiluted culture fluid.

(b) NI's against JBE virus were null in all cases.

Results appear to indicate: 1) live dengue virus of tissue culture origin stimulates production of specific neutralizing antibody in monkeys without exerting harmful effect when injected intracutaneously in relatively small amounts; 2) in such serological response, there is no practical difference between the groups inoculated with virus "undiluted" (i.e., of higher concentration) or virus "diluted" (i.e., of lower concentration); 3) formalin-treated virus has no antibody-producing activity, at least under the conditions adopted.

Reference: Hotta, S., 1957, Ann. Trop. Med. & Parasitol. 51, 249.

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

An arthropod tissue culture laboratory is being established, headed by Dr. Earl C. Suitor, Jr. Dr. Suitor was recently a guest of Dr. Max Day, C.S.I.R.O., Canberra, Australia, and Dr. Frank Fenner, Australian National University, for several months, learning methods of insect cell culture. The lines of insect cells in continuous culture, first initiated by T.D.C. Grace of Dr. Day's laboratory, are now being cultured at NAMRU-2. They will be used primarily for the purpose of arbovirus studies. It is hoped that these cells will prove to be a satisfactory experimental tool for the study of arboviruses as agents of arthropod infection.

During the 1963 epidemic season, JE virus was isolated from sentinel pigs. The viremia was measured and mosquitoes were infected by feeding upon the pigs. A high degree of mosquito susceptibility was demonstrated.

The level of antibody in pigs that will prevent infection of mosquitoes has been determined in view of the possibility of using vaccination as a means of breaking the pig-mosquito cycle. At a critical level of antibody, only a low grade viremia develops, too low to infect mosquitoes. Antibody subsequently rises to a high level.

REPORT FROM DR. GEORGE W. BERAN, SILLIMAN UNIVERSITY MEDICAL CENTER DUMAGUETE CITY, PHILIPPINES

We have now collected serum samples from seventy persons for serological study. All volunteers have been enrolled by family units and include only families with lifetime residence in Dumaguete City. Travel outside this city for more than one month at any one time by any one member of the family disqualified that family. Enrollment is continuing to bring the number of volunteers to 100. Each person receives a physical examination, and medical history is obtained.

Currently we are practicing the necessary techniques for hamster kidney cell culture and developing our mouse colony. We have HeLa and monkey kidney cultures continually.

Our preliminary plan is to assay these sera for neutralizing antibody levels against several arboviruses, including groups A, B, and unclassified. Quite definitely, we hope to include Japanese and Murray Valley as we lie between endemic areas of these two. We will probably also include yellow fever and, if possible, the Philippine hemorrhagic fever virus. We are currently in the middle of a clinical outbreak of hemorrhagic fever.

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

Human Serum Surveys for Langat (TP21) Viral Antibodies:

From 1961 to 1963, human serum surveys were conducted to detect possible natural infections with Langat virus. Out of 484 specimens obtained from (1) seven areas which were contrasting in ecology, (2) army personnel and anti-malarial laborers exposed to possible tick bits in various estates and jungles of Malaya, and (3) Ulu Langat residents and the police force trained in jungle operations in the Ulu Langat forest (see following table), 431 (89.0%) showed HI antibodies against TP21. However, no neutralizing antibody was demonstrated in 367 of these specimens tested. The HI antibody must therefore be due to cross-reacting antibody caused by other viruses in group B such as Japanese encephalitis and dengue. No evidence has thus been found of natural infection in man with Langat virus.

MALAYAN HUMAN SERA TESTED FOR LANGAT (TP21) ANTIBODY

SOURCE	HI TEST		NEUT. TEST	
	NO. TESTED	NO. POSITIVE (%)	NO. TESTED	NO. POSITIVE
Coastal swamp, Rantau Panjang	36	22(61%)	10	0
Kuala Lumpur Town	37	20(54%)	*N.D.	-
Aborigines, Bukit Lanjan, **F.R.	18	9(50%)	6	0
Coastal Rice plain, Bukit Meriam	50	22(44%)	16	0
Inland rice-valley, Kampong Terachi	48	19(40%)	6	0
Aborigines, Bukit Langong, **F.R.	21	7(33%)	2	0
Aborigines, Mountains	49	14(29%)	8	0
Totals	259	113(44%)	48	0
Army	98	91(92.8%)	98	0
Antimalaria laborers	127	127(100%)	127	0
Totals	225	218(96.9%)	225	0
Ulu Langat Residents	*N.D.		45	0
Ulu Langat Police Training Force	*N.D.		49	0
Totals			94	0
Grand Totals	484	431(89.0)	367	0

*N.D. = Not Done

**F.R. = Forest Reserve

Ecological Study of TP21 (Langat) Virus (Circulation Experiment).

The object of this circulation experiment is to determine the possibility of transmitting TP21 virus infection from *Rattus sabanus*, known to be one of the hosts of the tick vector, *Ixodes granulatus*, to the Ixodes ticks and back again to rats. The experiment is divided into 4 phases: (1) rough determination of the duration of viremia of sero-negative rats inoculated with TP21 virus, (2) a more precise determination of (1), (3) feeding of "clean" ticks on TP21 virus-infected rats during the period of viremia, and (4) feeding a number of recovered fed and possibly infected ticks on "clean" rats and inoculating others into suckling mice. Evidence of infection is determined by complement fixation test on the brain of the infected animal.

To date, the experiment is at phase 2 and results appear to indicate that the period of viremia falls between day 2 and day 10 after inoculation of the rat with the virus.

REPORT FROM DR. ALBERT RUDNICK
UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE
AND
THE INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR, MALAYSIA

Hemorrhagic fever cases, averaging about 4-5 per month, continue to occur in Penang Island. Fever, vomiting, abdominal pain, hemorrhagic signs, a marked thrombocytopenia, appearance of Turk cells, and collapse are among the prominent features of the disease. Mortality is about 10%. Cases are confined to the urban and semi-urban areas of Georgetown, where *Aedes aegypti* mosquitoes occur in relatively large numbers. Physicians in Penang assert that they have never observed this disease previously and feel that it is new to Penang. Eight viruses have been isolated from the acute-phase sera of patients. One isolate has been tentatively identified as dengue type 2. The remaining isolates are in process of mouse adaptation, but on the basis of dengue challenge tests, all belong to the dengue sub-group of viruses. Chikungunya virus does not appear to be involved as evidenced by negative serology for Chikungunya antibody.

A normal human serum survey is being conducted in relation to the H-fever in Penang. In addition, mosquito collections and collection of serum and tissue specimens from common domestic and wild animals in Penang have been made and these are being tested for presence of virus and/or antibody. Of the animal

sera tested for dengue antibody so far in Penang, only one has been positive for both HI and neutralizing antibodies, a house shrew, Suncus murinus, collected in the urban area. Outside of monkey and man, this is the only animal in Malaya shown to possess heat-resistant dengue neutralizing antibody in high titer. Suncus murinus is found in association with human dwellings, primarily in urban areas, and is considered an introduced animal. The possibility that it may be involved in a dengue cycle is of great interest.

The dengue ecology preliminary survey in jungle areas away from human activity is continuing. No isolations of dengue virus have yet been verified despite several suspicious passages that show partial resistance to dengue challenge. However, a high percentage of monkeys collected and transported to the laboratory in mosquito-proof cages have heat-resistant dengue neutralizing antibody. The survey is now being modified to place a greater emphasis on collection of species of animals not taken in large numbers to date. Special emphasis will be placed on Suncus-related species, including the use of sentinel Suncus in the bait traps.

During the course of these studies, other viruses have been isolated. All are in process of adaptation. These isolates are from Aedes albopictus, Culex sinensis, Rattus jalorensis tissues, domestic pig serum, and domestic chicken tissues.

REPORT FROM MAJ. SCOTT B. HALSTEAD, MAJ. JOHN E. SCANLON, AND DR. CHARAS YAMARAT, VIRUS AND ENTOMOLOGY DEPARTMENTS, SEATO MEDICAL RESEARCH LABORATORY AND SCHOOL OF PUBLIC HEALTH BANGKOK, THAILAND

Asian Mosquito-borne Hemorrhagic Fever Outbreaks in 1963:

As is undoubtedly described elsewhere in the Infoexchange, several outbreaks of dengue virus disease characterized by fever, cardiovascular collapse, hemorrhagic phenomena, non-icteric hepatitis, interstitial pneumonitis, serous effusions and appreciable mortality have occurred in widely scattered areas of South and Southeast Asia in 1963. Areas involved include Penang, Malaysia; Calcutta, India; Thailand, South Vietnam, and possibly Rangoon, Burma. Visits to each area except Malaysia were made by staff members of the SEATO Medical Research Laboratory.

Epidemiologically, disease in all areas resembles that in Thailand in that it was predominantly confined to children. However, in Calcutta, several "typical" hemorrhagic fever cases and deaths have occurred in young adults. If confirmed virologically, the outbreak in India changes two concepts of the disease which have derived from studies in Southeast Asia: 1) that the HF syndrome is age related, and 2) that Caucasians acquire typical dengue fever and not HF with dengue virus infections. Of interest has been the reported recovery and tentative identification of chikungunya virus from Calcutta by Dr. Fred Bang of Johns Hopkins and Dr. J.K. Sarkar of the Calcutta School of Tropical Medicine.

South Vietnam. The hemorrhagic fever outbreak in South Vietnam was of interest in several respects. First, the major recognized outbreak occurred in a rural area and not the city of Saigon. Beginning in May 1963, a severe disease of children accompanied by hemorrhagic phenomena and 33% mortality was noted in An Giang Province near the Cambodian border. By August, the similarities between this disease and Thai hemorrhagic fever were recognized. Approximately 350 cases with 116 deaths have been reported for the period May-November. Military insecurity in the area prevented direct investigation of this outbreak, but acute sera collected from 10 patients yielded 1 dengue virus isolate and 6 sera had high HI and CF titers to dengue. *Aedes aegypti* were collected from the area of the outbreak.

The second observation of interest was the discovery of at least 10 typical and serologically documented hemorrhagic fever cases in Saigon in November. These cases were discovered in hospitals in the absence of any recognized disease in that city. All cases appeared to be dengue related; and dengue virus has been isolated from *Aedes aegypti* in Saigon. This failure of health workers to recognize or report endemic hemorrhagic fever in a large urban area with modern hospital facilities, even when HF was considered a major problem elsewhere in the country is significant. This experience implies that endemic HF could have existed unrecognized for years in Saigon and any attempt to affix a date for the beginning of hemorrhagic fever would be only speculative.

Thailand. In the Bangkok and Thonburi hospitals in 1963, there were 1538 hemorrhagic fever patients hospitalized with 144 deaths.

Hemorrhagic fever meetings.

The SEATO Lab is sponsoring a working meeting of pediatricians, virologists, and public health workers in Bangkok, February 13-15, 1964, to exchange data on studies in Thailand.

WHO has tentatively scheduled an international symposium on the problem for October 1964.

Dengue Virus Isolation Methods.

Because of widespread interest in dengue in laboratories in Asia and the Americas, it appears that laboratories with past experience with this problem should detail their virus isolation methodology. This is suggested not only as an aid to laboratories newly working with dengue, but because comparison of isolation techniques in different dengue outbreaks may provide useful data about variations of biologic behavior of dengue viruses.

SMRL Isolation Protocol.

Using suckling mice, this laboratory has recovered nearly 150 dengue viruses of multiple antigenic types from humans and arthropods collected in Thailand and Vietnam over the past 2 years. Human sera are diluted 1:4 in PBS and inoculated 0.01 ml IC and 0.02 ml IP in 2 litters of 1-day-old mice. Mosquitoes are inoculated in a similar fashion after trituration in 0.75% BPA and centrifugation at 10,000 rpm for 30 minutes. Each ml of diluent contains 500 units penicillin, 500 µg streptomycin, and 500 ug kanamycin. Specimens are passed 3 times routinely. Brains are harvested 10 days after 1st and 2nd passage and 10^{-1} suspensions are made in 0.75% BPA for further passage. Dengue viruses are usually recovered on 2nd or 3rd passage with incubation periods ranging from 8-16 days. All mice in both litters are generally sick simultaneously and mouse brain seeds at 4th or 5th passage generally have titers in excess of 10^{-5}.

In criticism of the early blind passage technique, it has been suggested that long incubation period dengue viruses may be selected against and thus not recovered. This hypothesis has not been evaluated.

Recently, considerable success has been achieved in recovering dengue viruses in BS-C-1 (GMK) cells by an interference technique. Nearly 80 dengue viruses have been recovered from human and arthropod sources. The presence of virus is

recognized by the development of resistance in the cell sheet to a CPE producing virus (polio 1, chikungunya, ECHO 8 or 11 all may be used). Details of the methodology are too lengthy to be included in this communication but may be furnished upon request.

REPORT FROM DR. R.L. DOHERTY
QUEENSLAND INSTITUTE OF MEDICAL RESEARCH, BRISBANE

A total of 2368 mosquitoes collected in January 1959 by Dr. A.K. O'Gower, then of the School of Public Health and Tropical Medicine, University of Sydney, were inoculated into mice in May 1963. One virus (T48) only was isolated, from a pool of 88 Aedes (Ochlerotatus) vigilax. It has been shown to be a group A virus distinct from the two members of that group previously isolated in Australia (Sindbis and Getah) and from Bebaru. Certain properties of the virus (e.g. high virus titer in muscle and antigenic relationship to Getah and Bebaru) suggested a possible similarity to Sagiyama virus, and two strains of Sagiyama were obtained by the courtesy of Dr. W.F. Scherer, Cornell University Medical College. HI tests (Table 1) showed however that T48 was also clearly distinct from Sagiyama. T48 has been sent to Dr. M. Theiler, Rockefeller Foundation Virus Laboratories, for further study.

Shope and Anderson showed that some patients from an epidemic of polyarthritis with rash developed group A antibodies, and suggested that this syndrome, known from several outbreaks in Australia, might be caused by an undiscovered group A virus. We considered the possibility that T48 might be the cause of this disease. Four outbreaks of polyarthritis (at Cairns in 1961 and in Western New South Wales, Western Queensland, and the Brisbane area in 1962) were studied. Each had evidence of current group A infection. Antibodies were detected more frequently and in higher titer to T48 than to Getah, Sindbis, or Bebaru, suggesting that T48 was at least the most closely related of the four to the agent of epidemic polyarthritis and that it might be identical with it.

TABLE 1

COMPARISON OF FOUR GROUP A VIRUSES

BY HI TEST

Antiserum[1]	Antigen[2]			
	Getah (N544)	Sagiyama (A588)	T48	Bebaru (AMM2354)
Getah	640	320	20	10
Sagiyama (Mag 132)	320	320	40	10
T48	20	40	320	10
Bebaru	10	10	10	160

[1] mouse antisera

[2] antigens prepared by sucrose-acetone extraction of mouse brain (Getah, Sagiyama and Bebaru) or acetone-ether extraction of mouse carcass (T48).

REPORT FROM PACIFIC RESEARCH SECTION
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
HONOLULU, HAWAII

The Pacific Research Section, NIAID, was established to exploit some of the advantages of working on the epidemiology of infectious agents on islands in the Pacific area. It is well known that some of these islands are relatively isolated and that most have a relatively simple flora and fauna.

This Section is planning to undertake, among other projects, epidemiologic studies on arboviruses if areas can be found which offer unique advantages for such investigations. As a beginning, sera were collected from approximately 1800 persons residing in various parts of New Caledonia and on a number of islands in the New Hebrides. In these collections, an effort was made to secure specimens from persons who had never left their current place of residence and who were less than 20 years of age. The only arbovirus infection which is presently known to have occurred in this geographic area is dengue (which was present in epidemic form in 1943-44). It is planned to test the sera collected in HI tests against various viruses isolated in Southeast Asia, Australia, and New Zealand. If evidence of recent arbovirus activity is obtained, field studies will be undertaken in an attempt to study the situation further.

EDITORIAL NOTE

The Seventh International Congresses of Tropical Medicine and Malaria, held in Rio de Janeiro, Brazil, in September 1963, not only produced significant panel presentations and symposia on arboviruses and hemorrhagic fever and fostered meetings of the Pan American Health Organization on I. Arbovirus Problems in the Large River Basins of Equatorial South America, and II. Recent Arbovirus Epidemics in the Americas and Information Exchange Activities, but provided a previously unparalleled opportunity for arbovirologists from all over the world to meet each other for the first time and to visit the productive arbovirus research activities in Latin America. The special PAHO meetings which were organized by Dr. Martins da Silva with co-chairmen William F. Scherer and William C. Reeves were particularly stimulating in this regard. These all-day sessions on September 5 and 7 provided opportunities for social as well as scientific association. The Pfizer Company generously facilitated the important fellowship which the free international association of participants in this Information Exchange activity utilized for exchange of scientific and personal information. To document this friendly meeting and to remind those present and their colleagues of the persons in attendance and participating, a photomontage has been prepared separately for distribution with this issue of the Infoexchange. Additional copies are available from here. Copies of the report on the meetings are obtainable from the Pan American Health Organization (Ref 63.1, 15 October 1963) in Washington, D.C. The latest word regarding publication of the Arbovirus Symposia Proceedings is that they are being edited by Dr. Shope (Belem Virus Laboratory) and Dr. Bruno-Lobo (Instituto de Microbiologia, Rio de Janeiro) for separate publication soon.

As a closing note, many contributions for issue number ten of the Infoexchange have already been received. Deadline for contributions to the next issue will be July 31, 1964. A reminder will be sent to all of you in June.

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