Amapari, a New Virus of the Tacaribe Group from Rodents and Mites of Amapa Territory, Brazil.* (31182)

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The Tacaribe group(1) of arboviruses consists of 3 agents, Tacaribe(2) Junín(3,4) and Machupo(5), which are closely related by complement-fixation test but readily distinguishable by neutralization test(5,6). During 1964 and 1965, 5 strains of a new virus of this group were isolated near Serra do Navio, Amapá Territory, Brazil, in the course of a field program for the study of arboviruses undertaken by the Belém Virus Laboratory of the Instituto Evandro Chagas in cooperation with Indústria e Comércio de Minérios S.A. (ICOMI), a Brazilian mining company.

Serro do Navio village is situated on the Amaparí river, 1° N latitude and 52° W longitude, almost at the geographic center of Amapá Territory, which lies north of the mouth of the Amazon river. It is inhabited by 2,500 employees of ICOMI and a few hundred indigenes. The region is characterized by heavy tropical forest, periodically inundated, along the river banks; behind the banks the land rises sharply to heavily forested hills. Total rainfall in 1964 was 2,293 mm, with the rainiest months from January through June. Between the town hospital and the river there is a half-mile stretch of virgin forest, partially flooded in the rainy season, and this formed the area for mammal, bird and mosquito captures.

Materials and methods. Processing of field specimens. Mammals and birds were captured between May and August 1964 and again from November 1964 through March 1965. Field techniques have been described in detail(7). Mammals were trapped in hardwood traps, and birds were netted or rarely shot. Sentinel mice were exposed for 24 or 48 hours, and mosquitoes were collected on human bait and shipped alive to Belém by air. Animals were bled by cardiac puncture. During the initial part of the study, animals were sacrificed in the field and autopsied, and the heart, liver, kidneys and spleen were shipped in glycerin to Belém where the material was stored at —60°C until processed for virus isolation attempts. Later, it was found more practical to ship the animals alive in individual cages to Belém and to complete the other steps there. Virus isolation attempts were done in 2- to 3-day-old mice inoculated intracerebrally (i.c.)

Serological techniques. Complement-fixation (CF) testing was done by a technique already described(8) using sucrose-acetone extracted antigens(9) and immune sera prepared in mice or guinea pigs as well as hyperimmune mouse ascitic fluids(10).

In neutralization (N) testing in baby mice of the Belém stock, use of mortality as a basis for calculating titers was unsatisfactory because deaths occurred irregularly and the infective titer was greater than the death endpoint. Infective endpoints could, however, be readily determined after 7-10 days' incubation, using the presence of CF antigen in brain as the infectivity indicator. Accordingly, a N test was devised utilizing 2-day-old mice inoculated i.c. Baby mouse brain, harvested 6 days after infection, served as virus source. Sera were tested in the 1:4 final dilution without inactivation, and virus was used in 10-fold dilutions. Serum-virus mixtures were incubated for 1 hour at 37°C before inoculation. At 7 to 10 days mice were sacrificed, and brains were removed, frozen and

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thawed once and diluted in veronal buffer to make approximately a 1:20 dilution, the brain being triturated by aspiration through a 2-ml pipette several times. The suspension was centrifuged lightly and the supernatant fluid was reacted with homologous serum to determine the presence or absence of CF antigen. Titers, expressed as the log of the 50% infectious dose (log ID_{50}), were relatively high and endpoints clean and reproducible.

In contrast to mice of the Belém stock, mice of the Yale Arbovirus Research Unit stock were found satisfactory for N testing using mortality ratios as the basis for calculating endpoints. Hyperimmune sera were prepared in mice inoculated intraperitoneally (i.p.) with 0.3 ml of a 10^{-1} or 10^{-2} suspension of infected mouse brain; 4 injections were given for Tacaribe and 2 each for BE An-70563 and Junín viruses. Mice were bled 7-8 days after the last injection. These sera were lyophilized in 0.5-ml amounts; when needed for a test, 1 or more ampules were rehydrated and mixed in equal parts with an aliquot of a pool of normal mouse sera that also had been lyophilized in 0.5-ml amounts. Other aliquots of the same pool of normal mouse sera served as serum controls. Freshly passed virus, harvested 7 days after inoculation, was used in each test. Mixtures of equal volumes (0.25 ml) of virus in serial 10-fold dilutions and serum at constant dilution 1:2 were incubated in a water bath for 1 hour at 37°C and then inoculated i.c. in 0.02-ml amounts into groups of 8 mice 4-5 days old. The mice were observed for 3 weeks in the case of Junín virus, and for 4 weeks with Tacaribe and BE An-70563.

Results. Isolation of virus. BE An-70563 virus, the prototype strain, was isolated from a pool of liver, spleen, kidney and heart of a young adult male *Neacomys guianae* (Thomas, 1905), which was captured on July 8, 1964 near Serro do Navio and sacrificed the following day in Belém. Three more strains, closely related to BE An-70563 in CF testing, were isolated from pooled viscera of 3 adult male *Oryzomys* (probably *goeldii*) that were captured at the same site between Dec. 23, 1964 and Jan. 13, 1965, and held in captivity in Belém for 26, 26 and 5 days, respectively. Virus was not recovered from serum of these 4 animals. Reisolation from viscera was not attempted. A fifth strain of the virus, also closely related to BE An-70563 in CF testing, was isolated from a pool of 199 mites† combed in Jan. 1965 from 8 *Oryzomys,* including 2 that subsequently yielded virus This last strain was reisolated from the stored mite pool suspension.

TABLE I. Virus Isolation Attempts with Forest Animals of Serra do Navio and Number of Strains of Amaparí Virus Recovered, 1964-1965.

Animal	No. viruses isolated/No. viscera pools inoculated: Viscera preserved in glycerin	Animals shipped alive to laboratory	No. viruses isolated/No. sera inoculated
Marsupial	0/160	0/5	—
Rodent			
Proechimys	0/60	0/6	0/7
Oryzomys	0/30	3/23*	0/28
Neacomys	0/46	1/7 †	0/9
Other	0/4	0/3	0/3
Bat‡			
Macrophyllum	0/5	—	—
Anoura	0/7	—	—
Carollia	0/16	—	—
Not identified	0/49	0/8	0/8
Bird	0/405	—	—
Other animal	0/26	—	—

* Strains BE An-81087, BE An-81088 and BE An-81092.

† Strain BE An-70563, the prototype.

‡ With bats, salivary glands were tested in addition to other viscera.

No other Tacaribe group virus strains were recovered from the 862 forest mammals and birds tested (Table I) or from arthropod sources, consisting of 7,054 mosquitoes, 2,391 mites and 346 other ectoparasites. None of 120 families of sentinel mice exposed in the study area yielded virus.

Characterization of the isolates. Although many baby mice survived i.c. inoculation of strain BE An-70563 in initial mouse brain passages, by the 4th passage a 10% brain suspension of the virus killed 3-day-old mice of the Belém stock inoculated i.c. and i.p. with average survival times of 13.7 and 18.0

† Mites taken from 2 *Oryzomys* captured at the same site were identified as *Gigantolaelaps oudemansi* and *Haemolaelaps* sp. by Dr. Conrad E. Yunker, Rocky Mountain Laboratory, Hamilton, Mont.

TABLE II. Cross-CF Testing with the BE An-70563 Strain of Amaparí Virus and Tacaribe and Junín Viruses.

Antigen	Hyperimmune mouse serum		
	BE An-70563 2 injections	Tacaribe 4 injections	Junín 3 injections
BE An-70563	128/256*	32/64	32/64
Tacaribe, TRVL 11573	32/128	256/256	64/128
Junín, XJ	64/128	64/64	128/128
Normal brain	0/0	0/0	0/0

* Titers are expressed as reciprocal of serum dilution over reciprocal of antigen dilution.

days. Adult mice survived both i.c. and i.p. inoculation and developed CF antibody. At the 7th passage the i.c. 50% lethal dose (LD_{50}) for mice was 2.5 log and the i.c. ID_{50}, using CF antigen in brain as the infectivity indicator, was 6.7 log. The i.p. ID_{50} of the same inoculum was equal to or less than 2.5 log. In baby mice inoculated i.c. viremia was detected on postinoculation days 2-6, the highest titer being noted on day 6 when the ID_{50} was 1.7 log. Testing for viremia was discontinued after day 6.

Guinea pigs inoculated i.p. with 5th mouse brain passage virus survived and developed CF antibody.

No cytopathic effect was observed in monolayer tube cultures of HEp-2, green monkey kidney (*Cercopithecus aethiops*) and BHK-21 (baby hamster kidney) continuous cell lines inoculated with strains BE An-70563 and BE An-81087 of Amaparí virus. Addition of 0.1, 1.0 and 10.0 μg of Hydrocortamate per ml of maintenance fluid had no apparent effect. When strain BE An-81087 was inoculated at the time of seeding flasks with cells, there were slight cell alterations after 2 weeks in the green monkey kidney and HEp-2 cultures, and the supernatant fluids of tubes inoculated with the 10^{-4} dilution contained CF antigen. No plaques were produced in chick embryo cultures with agar overlay.

Identification of virus. In initial CF testing in Belém, BE An-70563 antigen reacted with hyperimmune mouse ascitic fluid and serum of Tacaribe virus (TRVL 11573) and with immune guinea pig serum of Junín virus (XJ). At the Yale Arbovirus Research Unit, the results of further CF studies confirmed the new isolate's relationship to Tacaribe and Junín and also indicated that it differed from both (Table II). In cross-N tests done in baby mice, BE An-70563 was clearly distinguishable from the other 2 viruses (Table III).

In further testing done by a plaque neutralization technique(11) in Vero cells, Dr. Patricia Webb of the Middle America Research Unit showed that BE An-70563 virus differs from Machupo virus as well as from Tacaribe and Junín (Table IV).

On the basis of these results, BE An-70563 virus is considered a new member of the Tacaribe group. It is proposed to name the agent Amaparí virus after the river that flows through the Serra do Navio area.

Antibody survey. Eighty-seven sera of mammals captured at Serra do Navio were tested for CF antibody to Amaparí virus. One of 4 *Neacomys* sera reacted in the 1:4 dilution and 2 of 18 *Oryzomys* sera reacted in the 1:16 dilution. No reactions were observed with the remaining 23 *Proechimys*, 16 bat and 26 marsupial sera. Twenty-four sera of rodents from the Utinga forest near Belém were also CF negative. Three rodent sera from Serra do Navio, including the 2 CF positive sera from *Oryzomys*, had log neutralization indices of less than 0.4 for Ama-

TABLE III. Cross-N Testing with the BE An-70563 Strain of Amaparí Virus and Tacaribe and Junín Viruses.

Serum	Virus					
	BE An-70563		Tacaribe		Junín	
	Log LD_{50}	Log NI*	Log LD_{50}	Log NI	Log LD_{50}	Log NI
BE An-70563	*3.4*	*1.6*	4.8	.6	>6.5	<.5
Tacaribe, TRVL 11573	4.8	.2	*3.4*	*2.0*	>6.5	<.5
Junín, XJ	4.7	.3	4.7	.7	*4.0*	*3.0*
Normal serum	5.0		5.4		7.0	

* NI = neutralization index.

TABLE IV. Comparison of the BE An-70563 Strain of Amaparí Virus with Tacaribe, Junín and Machupo Viruses by Plaque N Test in Vero Cell Culture, Using 80% Reduction as Criterion of Positivity.*

A. Fixed virus—Varying serum dilution test†

Hyperimmune serum	Serum dilution endpoint with fixed virus dose of			
	BE An-70563	Tacaribe	Junín	Machupo
BE An-70563	1024	<4	<4	<4
Tacaribe, TRVL 11573	<4	2048		
Junín, XJ	<4		256	
Machupo, prototype	<4			128

B. Fixed serum—Varying virus dilution test, using BE An-70563 hyperimmune mouse serum 1:10

Virus dilution	No. virus plaques			
	BE An-70563‡	Tacaribe	Junín	Machupo
4	0	TNTC§	TNTC	TNTC
64	0	25+	TNTC	TNTC
1,024	0	4	13	TNTC
16,384	0	0	2.7	21
262,144	0	0	0	2

* These studies were performed by Dr. Patricia A. Webb, Middle America Research Unit, Canal Zone.

† Number of plaque-forming units (PFU) ranged from 10^1 to 10^8. Sera were diluted in 10% normal human serum, virus in phosphate-buffered saline with 2% chick serum. Virus-serum mixture was incubated for 1 hr at room temperature prior to adsorption for 1 hr at 36.5°C in a humidified atmosphere of 4% CO_2 and agar overlay. Second staining overlay with neutral red was added on day 5.

‡ BE An-70563 virus titered 2-3 PFU at 10^{-5} dilution.

§ TNTC = too numerous to count.

pari virus. Twelve sera of adult human residents of Serra do Navio neutralized less than 1.3 log ID_{50} of the virus.

Discussion. With the addition of Amaparí virus, the Tacaribe group now consists of 2 members that have been implicated in human disease and 2 that have not. Junín and Machupo viruses have been isolated repeatedly from nonfatal and fatal cases of Argentinian hemorrhagic fever(3,4) and Bolivian hemorrhagic fever(5), respectively. Hemorrhagic fever has not been reported in Amapá Territory, Brazil, and there is no evidence that Amaparí virus infects man. Nor is there any evidence that Tacaribe virus, isolated from bats and once from mosquitoes(2), infects human beings. Bats of genera *Artibeus* and *Desmodus* infected by inoculation with Tacaribe in the laboratory did not circulate virus, but virus was recovered from organs of several bats; detectable antibody was shown in only 1 bat of 39 studied(2). Junín virus has also been recovered from rodents(12) and rodent ectoparasites(13,14), and long-term excretion of Machupo virus in the urine of infected rodents has been demonstrated(15).

N testing with the presence of CF antigen in mouse brain used as the infectivity indicator gave satisfactory results with experimentally prepared immune sera. Thus far, neutralizing antibody in survey sera of human beings and wild animals has not been detected by this technique, and the test's usefulness must await further experience.

Summary. Amaparí virus is a new member of the Tacaribe virus group which has been isolated on 5 occasions at Serra do Navio, Amapá Territory, Brazil, during 1964 and 1965. One strain was recovered from organs of *Neacomys,* 3 from organs of *Oryzomys* and 1 from mites combed from *Oryzomys.* The new virus is closely related in complement-fixation (CF) testing to the other 3 members of the group—Tacaribe, Junín and Machupo—but differs from them in neutralization testing. Limited studies revealed no neutralizing antibody in sera of human residents of Serra do Navio. *Oryzomys* captured in the area had CF but not neutralizing antibody.

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Influence of Diet and Heredity on the Serum Protein Components of The Rat. (31183)

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One of several criteria used in this laboratory to determine the nutritional response of the rat to different diets is the measurement of the concentration of the various serum protein fractions. Heredity is also known to be a major factor affecting the distribution and concentration of the blood proteins(1). With the increased application of the more definitive electrophoretic techniques, genetic variations in almost all the serum protein components have been established(2).

Marshall and Hildebrand(3) have shown that such measurements as body growth, food intake, urinary protein, organ weights, and body and liver composition are influenced by the strain of the rat as well as by the diet. The differences observed in liver and body composition among the 3 strains examined indicated that the response to diet must be related to differences in inherent metabolic characteristics, particularly the mechanism of lipid synthesis. The effect of level and kind of dietary fat on the serum protein components of one of these strains has been reported(4) and the component most affected was pre-albumin, a lipid-protein fraction migrating more rapidly than albumin. In this paper are reported the differences observed