occurred between Col SK virus and poliomyelitis or Coxsackie Group B virus on HeLa cells, causing suppression of about 1 to 3 logs of challenge virus depending on the dose of interfering virus used. 3) The mechanism of the interference reaction is discussed.

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Bussuquara, A New Arthropod-Borne Virus.* (24909)

GILDA GOMES† AND OTTIS R. CAUSEY (Introduced by J. Casals)
Rockefeller Foundation Virus Lab., N. Y., and Belém Virus Lab., Belém (Pará), Brazil

Sentinel monkeys have been used extensively by the Belém Virus Laboratory in attempts to isolate arthropod-borne (arbor) viruses. In this paper are described serologic studies that led to characterization of an agent (An 4073) thus isolated as a new, hitherto undescribed arbor virus, which it is proposed to name Bussuquara. *Circumstances of isolation and early studies.* Bussuquara virus was isolated in the forest of the Inst. Agronomico do Norte, Belém. Blood from a sentinel howler monkey (*Alouatta beelzebul*) was inoculated intracerebrally into 3-day-old mice. Beginning on sixth day, some inoculated mice showed signs of illness. Brain tissue emulsions from these sick animals were in turn pathogenic for new suckling mice on intracerebral inoculation. The agent isolated was serially propagated by intracerebral passage in suckling mice. It readily passed through Seitz filters, and cultures of the infectious brain emulsions proved sterile on bacteriological media. Three days later the virus was again isolated from blood of the monkey. Sixteen days after second sample of infected blood was taken, the monkey died, and as serum from the moribund animal was markedly icteric, the liver was submitted to Dr. Madureira Para for histopathological examination. He reported lesions compatible with a diagnosis of yellow fever. Although the isolated virus was not neutralized by a known yellow fever immune serum, serum samples from adult mice that had survived intracerebral inoculation of this virus reacted in a hemagglutination-inhibition (HI) test with several antigens of group B arbor viruses at dilutions up to 1:160. To study the immunologic relationship of the agent further, the following experiments were made.

Methods. Immunologic characterization. No special effort was made to determine biological properties of the agent beyond what was necessary to characterize it

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† Present address: Inst. de Microbiologia, Rio de Janeiro, Brazil.

serologically.‡ In second to fifth mouse passages, the virus was pathogenic for newborn mice on intracerebral and intraperitoneal inoculation with a titer around 10^{-8}, and for adult mice on intracerebral inoculation with a titer of approximately 10^{-7}. Average survival time of newborn mice was 4 or 5 days and that of young adults around 6 days, when an inoculum of 10^{-1} or 10^{-2} dilution of virus was used. Immunologic characterization of the virus was done by HI, complement-fixation (CF) and neutralization tests. Procedures for the HI test have been described(1). Immune sera were prepared by inoculation of the respective viruses either once or repeatedly from 3 to 6 times. All multiple-injection sera, as well as single-injection sera against Bussuquara and bat salivary gland (BSG) viruses, were obtained from mice after intraperitoneal inoculation; the remaining single-injection sera were obtained from guinea pigs inoculated intracerebrally. Except when otherwise indicated, acetone-ether extracted antigens were used in HI and CF tests. In CF tests, extensive use was made of box- or checker-board-titration system, in which each serum was tested in increasing 2-fold dilutions beginning at 1:4 (occasionally 1:2) and extending to 1:512 against each antigen similarly diluted. Our results of box-titration CF test are expressed as fractions, the numerator being titer of serum and the denominator, titer of antigen. Neutralization tests were carried out by intracerebral inoculation into 3- to 6-day-old mice of mixtures of equal parts of undiluted serum and dilutions of virus. Mixtures were incubated at 37°C for 1 hour before inoculation.

Results. Extracts to be used as antigens in a CF test were prepared from brain and from liver tissue of newborn mice infected with Bussuquara virus by centrifugation at 2000 rpm for 20 minutes of a 10% emulsion of each tissue in physiological salt solution. The supernatant fluids, designated undiluted antigens, were tested in dilutions 1:2, 1:8 and 1:16 against 4 serum pools, each in 2-fold dilutions ranging from 1:2 to 1:64. These 4 pools contained equal parts of hyperimmune mouse serum against individual arbor viruses as follows: pool 1—(group A) Mayaro, Venezuelan equine encephalitis, western equine encephalitis and eastern equine encephalitis; pool 2—(group B) Ilhéus, dengue type 1, yellow fever and Zika; pool 3—(group C) Oriboca, Murutucu and Apeu; and pool 4—(miscellaneous) Bunyamwera, Be An 277,§ Be H 151 and Tr 8900. Of the 8 antigen-serum combinations, only one reacted positively, namely, serum pool group B and brain tissue extract; the serum gave a titer of 1:8 and the antigen reacted at a dilution of 1:2. This result confirmed the earlier indication that Bussuquara is a group B virus.

Additional evidence was secured by further HI tests. Hemagglutinating antigens with Bussuquara were prepared from infected brain tissues of suckling mice. The antigen was active at pH range of 6.4 to 7.6, with optimal zone between 6.8 and 7.3; as a rule the antigen was used at pH 7.0 and incubated at either 22°C or 37°C. Under these conditions and on lucite trays, titer of antigen was of the order of 1:300. Twelve known immune mouse sera against 12 arbor viruses were tested simultaneously with 4 or 8 units of Bussuquara antigen and with their homologous antigens; 7 of the sera represented group A arbor viruses, 4 sera group B agents and one serum Bunyamwera virus. The results of this test were as follows, homologous titer being given first: Group A—Chikungunya, 1:160, 0; eastern equine encephalitis, 1:320, 0; Mayaro, 1:640, 0; Semliki Forest, 1:2560, 0; western equine encephalitis, 1:1280, 0; AMM 2021, 1:640, 0; AMM 2354, 1:320, 0; Group B—BSG, 1:160, 1:80; St. Louis encephalitis (SLE), 1:80, 1:20; West Nile (WN), 1:640, 1:160; yellow fever, 1:640, 1:320; and finally, Bunyamwera, 1:640, 0.

‡ Bussuquara virus has been maintained serially by inoculation of mosquitoes, thus fulfilling one of the essential experimental criteria for arthropod-borne nature of a virus (Dr. Loring Whitman, personal communication).

§ Some viruses mentioned in this paper have not yet been described by the authors responsible for their isolation: Be An 277 and Be H 151, isolated by Dr. Ottis R. Causey; Tr 8900, by Drs. Wilbur G. Downs and Charles R. Anderson; AMM 2021 and AMM 2354, by Dr. E. L. Buescher; and SA H 336, by Dr. Kenneth C. Smithburn.

TABLE I. Complement-Fixation Test.

Serum	Antigen				
	Buss.	SLE	Ilhéus	BSG	SA H 336
Bussuquara	1:64/1:16	1:8/1:32	1:4/1:16	0/0	1:4/1:8
St. Louis encephalitis	1:4/1:4	1:128/1:256			
Ilhéus	0/0		1:256/1:512		
Bat salivary gland	1:4/1:2			1:64/1:64	
SA H 336	0/0				1:32/1:256

These results leave no doubt that Bussuquara virus belongs in group B of the arbor viruses.

With the group affiliation of Bussuquara virus thus established, attempts were made to determine whether it was a new agent, *i.e.*, distinguishable from previously described group B viruses. For this the CF test was used extensively, since previous experience had shown it to be in general more specific than HI in group B(2). Multiple-injection mouse sera were used and, with one exception to be noted later, complete box titrations as described above were done. In the 3 complete box-titration tests carried out several antigen-serum systems were included in addition to Bussuquara, which was present in all; samples of Bussuquara antigen and serum were of different lots in the different tests. In the first test, the homologous titers of Bussuquara serum and antigen were 1:16 and 1:8, respectively; in the other 2 tests, 1:64 and 1:16. In no instance did Bussuquara antigens or sera react with antigens or antisera prepared from yellow fever (French neurotropic strain), dengue type 1, dengue type 2, Japanese encephalitis or WN (Egypt 101 strain) viruses, even though these systems were shown to have titers of 1:32 to 1:128 for the sera and 1:64 to 1:256 for the antigens.

Table I gives results obtained when Bussuquara antigens or sera were tested against similar reagents prepared from SLE, BSG, Ilhéus and SA H 336 viruses. It can be seen that sera from mice immune to these 4 viruses either failed to react with Bussuquara antigen, or at best reacted to 1/16 or less of their homologous titer, even then only in the presence of an excess of antigen. Similarly, Bussuquara virus immune serum was at best but weakly reactive with the other antigens and again only when these were in excess quantity.

Cross reactions of the type shown in Table I could not be detected in all cases when the systems were tested owing probably to the low titers of the cross reaction. Thus, in 4 tests with 2 different SLE immune sera, only the positive cross reaction shown in Table I occurred, and in 3 tests with 2 different BSG immune sera there was also only one positive cross reaction. On the other hand, all 4 different samples of Bussuquara virus immune serum reacted with SLE antigen, but only one sample of 2 tested reacted with Ilhéus and SA H 336 antigens.

An additional CF test was carried out to compare Bussuquara virus with other group B agents not included in previous tests. In this test, Bussuquara antigen was used in dilutions 1:2.5, 1:5, 1:10 and 1:20; all other antigens in dilutions 1:8 and 1:16. Immune sera were used in serial 2-fold dilutions beginning at 1:2 for Bussuquara and at 1:4 for all the other viruses. The Bussuquara immune serum titrated 1:16 and the antigen 1:10. Homologous titers for the other immune sera were: Uganda S, 1:32; Zika, Russian spring-summer encephalitis and Spondweni, 1:64; and Japanese encephalitis, Ntaya and Wesselsbron, 1:128. Only one of these sera, Spondweni, reacted with the Bussuquara antigen and then only in a titer of 1:4. The Bussuquara serum failed to react with any of the antigens included in the test. While it is possible that some cross reactions might have been demonstrable had sera or antigens been more potent, the results of the CF tests show conclusively that Bussuquara virus is antigenically distinct from any of the other group B viruses included in the tests.

Studies by HI did not separate Bussuquara virus as sharply from other group B viruses as did CF tests, particularly when the immune sera used were from mice repeatedly inoculated. Since it has been reported(2) that sera

TABLE II. Hemagglutination-Inhibition Test.

Serum	Antigen					
	Buss.	BSG	Ilhéus	SLE	WN	JE
Bussuquara	160*	10	40	40		
Bat salivary gland	80	160				
Ilhéus	160		1280			
St. Louis encephalitis	10			640		
West Nile	10				320	
Japanese encephalitis	10					160

* 160, titer of serum is 1:160.

TABLE III. Summary of 4 Intracerebral Neutralization Tests.

Virus	No. of inoc.	Neutralization indices: Serum, immune to—Virus			
		Buss.	SLE	BSG	Ilhéus
Bussuquara	3	400	1	80	10
St. Louis encephalitis	5	<3	630		
Bat salivary gland	6	10		100,000	
Ilhéus	5	30			1000
Japanese encephalitis	5	5			

from animals bled within 7 to 10 days after a single injection of virus are likely to show a higher degree of specificity than sera obtained after multiple inoculations, some such sera were used for the characterization of Bussuquara virus. From the results (Table II) it can be seen that Bussuquara virus is readily separable from the other agents in the test.

Studies by neutralization test were not as extensive as those done by HI and CF tests, and were limited to viruses and sera with which Bussuquara virus had shown the highest degree of cross reaction in *in vitro* tests. Results of 4 neutralization tests are summarized in Table III.

In these tests, aliquots of a sample of each of 5 immune sera were tested for capacity to protect suckling mice against Bussuquara virus and against each of the homologous viruses with the exception of Japanese encephalitis virus, which was not tested. In addition, Bussuquara virus immune serum was tested against the homologous virus and 3 others. The results of these tests showed clearly that Bussuquara virus is easily distinguishable from SLE, Ilhéus, BSG and Japanese encephalitis viruses. In fact, except for overlap between Bussuquara serum and BSG virus, there was no significant cross reaction.

Summary and conclusions. A virus strain, An 4073, was isolated from blood of a sentinel howler monkey near Belém (Pará), Brazil. This virus yielded a hemagglutinating antigen without difficulty. By means of CF and HI tests with sera from mice immunized with repeated injections, this strain was shown as a member of group B of arthropod-borne viruses. Further studies, in which, in addition, single-injection immune sera and neutralization test were employed, gave evidence that the new agent could be easily distinguished serologically from 14 different group B viruses. Other group B viruses, such as Murray Valley encephalitis and louping ill, with which Bussuquara was not compared are so close to some of those studied that their inclusion in this investigation was considered unnecessary. The limited cross reactions obtained in CF tests between Bussuquara virus and other group B agents indicate a distant relationship. While the negative results may be attributed, in part, to low titers of Bussuquara sera and antigens, more potent systems would still be expected to show the same relative cross reactivity. In view of our results, it is concluded that the strain of virus studied constitutes a new, hitherto unreported arthropod-borne virus belonging in group B. The

virus has been given the name Bussuquara.

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Comparative Response of Normal and Cirrhotic Rats to Intravenously Injected Bacteria.* (24910)

ALEXANDER M. RUTENBURG, EDMUND SONNENBLICK, IRVING KOVEN, FRITZ SCHWEINBURG AND JACOB FINE

Depts. of Surgery, Harvard Medical School, and Beth Israel Hospital, Boston

During study of role of intestinal bacteria in genesis of experimental dietary cirrhosis in rats(1), we noted abscesses in the lung or liver of rats which developed cirrhosis and, by contrast, the absence of such infection in those protected from developing cirrhosis. This suggests that the cirrhotic rats were less resistant to common bacterial invaders. In this communication we report results of a study of antibacterial resistance of these cirrhotic rats.

Method. The experiments were designed to compare ability of normal and cirrhotic rats(1) to clear the circulation of bacteria injected into the portal vein; and (2) to lyse the ingested bacteria. *E. coli,* labelled with I^{131} by method previously reported(2), were washed in saline (0.85%) and then dialyzed against saline at room temperature until almost all radioactivity was removed from supernatant. Assay of the supernatant of the bacterial suspension for radioactivity at various intervals during 48 hours after dialysis, demonstrated that the iodine taken up remained tightly bound. All radioactivity determinations were done with deep well scintillometer. The different bacterial suspensions injected were adjusted by dilution to yield approximately the same concentration of bacteria and were about equal in terms of radioactivity. The viability of these labelled bacteria was tested by subculture every 12 hours. It was estimated from colony counts that all remained viable for duration of experiment.

Normal and cirrhotic† male albino rats of the Wistar strain (wt. 250-300 g) received 10 drops of saturated solution of KI in their drinking water each day for 3 days prior to experiment to saturate the thyroid as well as other tissues with iodine, and thus preclude binding of liberated I^{131}. Through a small midline abdominal incision made under light ether anesthesia, 0.5 ml of a bacterial suspension (10^8 bacteria) of I^{131} labelled *E. coli* was injected into the splenic vein of each rat. The same volume of this suspension was used as standard to measure total radioactivity injected. Smaller injectates gave poorly reproducible curves; larger injectates were rapidly fatal to cirrhotic rats.

Each rat was isolated in individual metabolic cage and all excreta collected. At various intervals after injection the rats were killed by exsanguination. One gram samples of blood, liver, spleen, lung, and urine were planted in various media for cultures of aerobic and anaerobic bacteria. Samples of cirrhotic livers were taken for microscopic confirmation of cirrhosis. Liver, lungs, spleen, kidneys, gastrointestinal tract, thyroid, heart, and a portion of muscle (hamstring) were removed, weighed and homogenized. Appropriate aliquots (2 ml) of the homogenates were then assayed in duplicate for gamma radiation by deep well scintillometer. Remainder of homogenate was then dialyzed at room temperature against running tap water for 24 hours, after which 2 ml aliquots were measured for gamma radioactivity. The total, bound and dialyzable radioactivity

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† Cirrhosis was induced by feeding choline deficient diet for 300 days(1).