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THE USE OF THE IMMUNE-ADHERENCE REACTION IN THE STUDY OF SOME BRAZILIAN ARBOVIRUSES

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SUMMARY

The immune-adherence test was utilized to study the serological relationships of viruses within arbovirus groups A, B, C, and Guamá, and complex Capim. A comparison of complement fixation and immune-adherence techniques using viruses of groups A, B, and C showed that the immune-adherence test is the more sensitive, but that both the immune-adherence and the complement fixation tests are of nearly equal specificity. The immune-adherence antigen for Guamá virus was found in higher concentration in the liver than in the serum or brain of infected newborn mice. The complement fixation and immune-adherence activity of this liver preparation were not separated by DEAE-cellulose column fractionation.

INTRODUCTION

The adherence reaction was noted as early as 1901 by LEVADITI [7] who observed the attachment of *Vibrio cholerae* to platelets in the bloodstream, when these microorganisms were inoculated into previously immunized guinea-pigs.

Since then, this phenomenon has been studied in many different antigen-antibody systems with various techniques. LAMMANA [6] reviewed the technical improvements introduced to demonstrate the reaction and the different names given to the test. NELSON'S [8] designation "immune-adherence" (IA) reaction has been adopted in the present study.

NELSON & WOODWORTH [9] showed that IA could be revealed by hemagglutination, and TAVERNE [13] demonstrated the adherence of bacteriophages of the T_2 strain to erythrocytes, pointing out that readings should be immediate since the phenomenon disappears in a short time.

ESTEVES [2] compared the IA and the complement fixation (CF) tests in the typing of foot-and-mouth disease viruses and observed that the IA test was the more sensitive. ESTEVES et al.[3] used the IA reaction to study group A arboviruses isolated in Brasil.

In the present work the feasibility of extending the use of the IA test to a larger number of arthropod-borne viruses was explored. Viruses of arbovirus complexes or groups A, B, C, Guamá, and Capim, which constitute a large and important part of the arboviruses known in Brasil, were used in this investigation.

MATERIALS AND METHODS

Viruses — All strains utilized were originally isolated at the Belém Virus Laboratory. The names and strain numbers are as follows:

Group A — eastern equine encephalomyelitis (EEE), AN 7526; Mucambo, AN 10967; Mayaro, AR 20290; Aurá, AR 10315; and Pixuna, AR 35645.

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Group B — yellow fever (YF), H 111; Bussuquara, AN 4116; Ilheus, H 7445; and St. Louis encephalitis (SLE), AR 23379.

Group C — Marituba, AN 15; Oriboca, AN 17; Apeú, AN 848; Murutucú, AN 974; Caraparú, AN 3994; and Itaqui, AN 12797.

Group Guamá — Guamá, AN 277; Catú, H 151; and Mojú, AR 12590.

Capim complex — Capim, AN 8582; Guajará, AN 10615; and Bush Bush, AN 20076.

Mirim * — Mirim, AN 7722.

Sera — Hyperimmune sera prepared in mice by four or five intraperitoneal inoculations were employed. For viruses that kill adult mice, the first two inoculations were made with formalin inactivated material.

Bivalent sera (produced by immunization with two viruses in the same group) for group B viruses, and bivalent and trivalent sera for group A were prepared. In these cases, the mice were inoculated at 7 day intervals and bled 10 days after the last inoculation.

Antigens — Antigens were prepared with brain tissue, liver tissue, or blood serum from infected newborn mice. In the first two cases, 10% suspensions (weight/volume) were considered as undiluted antigens. For IA tests, the suspension was prepared in veronal buffered saline plus 0.5% bovine serum albumin (BA). The serum was utilized undiluted. Such antigens are termed "crude antigens". The brain and liver antigens called "purified" were prepared by the sucrose-acetone extraction technique of CLARKE & CASALS [1], and the "purified" serum antigens were extracted twice with acetone.

Red blood cells — Human erythrocytes from a single donor were used throughout. The blood was preserved in equal volumes of Alsever's solution. The cells were washed three times and suspended to 0.75% in BA and then adjusted so that hemolysis in 9 volumes of distilled water would give an O.D. of 0.160 in a Coleman Junior Spectrophotometer at a 450 mμ wave length in tubes of 10 mm internal diameter.

IA tests — IA testing was done by block titration, the reagents being distributed in plastic plates with V-shaped wells as described by SEVER [10]. The following sequence and volumes was observed:

Serum dilution	1 drop of 0.025 ml
Adequate dilution of complement	1 drop of 0.025 ml
Antigen dilution	1 drop of 0.025 ml
Erythrocyte suspension ...	2 drops of 0.025 ml

The reagents were incubated for one hour in a warm-air incubator at 37°C after the distribution of the first three components. A further incubation at 37°C for 30 minutes followed the addition of the erythrocytes. The plates were then transferred to a refrigerator at 4°C and the readings were performed as soon as the red blood cells in the controls had sedimented (approximately 10 minutes). Readings *4* and *0* were given respectively, to the wells where total or no agglutination occurred, the intermediate values being *1*, *2* and *3*.

The complement dilution taken as adequate was that developing the highest antigen titer against a constant serum dilution without non-specific agglutination. The complement dilution was usually about 1:60.

CF tests — CF testing was done by a microtechnique modified from FULTON & DUMBELL [4] using 2 units of complement and primary incubation overnight at 4°C.

Fractionation on diethylaminoethyl (DEAE) — cellulose column — The fractionation of Guamá virus was made according to the technique of KLEMPERER & PEREIRA [5] with liver tissue from infected newborn mice. Thirty-two fractions were collected using 0.1 M to 1.0 M saline solutions. Each fraction was tested against a constant dilution of hyperimmune serum by IA and CF. The serum was employed at the 1:80 dilution for IA tests and at the 1:4 dilution for CF tests.

* Mirim virus and the viruses of the Capim complex and their serological relationships have not appeared in formal publication and this mention is not intended to constitute such publication

TABLE I

Immune-adherence test. Guamá complex

SERA / ANTIGENS		X / XX	GUAMA 20	GUAMA 40	GUAMA 80	GUAMA 160	GUAMA 320	GUAMA 640	CATU 20	CATU 40	CATU 80	CATU 160	CATU 320	CATU 640	MOJU 20	MOJU 40	MOJU 80	MOJU 160	MOJU 320	MOJU 640	AC
GUAMA	CRUDE BRAIN	40	4	4	4	3	2	2	4	4	3	2	0	0	4	4	3	3	0	0	0
		80	4	4	3	0	0	0	4	3	3	1	0	0	4	4	2	1	0	0	0
		160	3	3	0	0	0	0	3	3	1	0	0	0	4	3	0	0	0	0	0
		320	3	2	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
		640	3	2	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0		0
	CRUDE LIVER	40	4	4	4	4	4	4	4	4	4	2	0	0	4	4	4	4	3	0	0
		80	4	4	4	4	4	4	4	4	4	1	0	0	4	4	4	4	3	0	0
		160	4	4	4	4	4	4	2	4	2	0	0	0	4	4	4	4	3	0	0
		320	4	4	4	4	4	4	2	2	0	0	0	0	4	4	4	4	3	0	0
		640	4	4	4	4	4	3	0	0	0	0	0	0	4	4	4	4	1	0	0
	PURIFIED SERUM	40	4	4	0	0	0	0	0	0	0	0	0	0	4	3	2	0	0	0	0
		80	4	4	0	0	0	0	0	0	0	0	0	0	4	2	0	0	0	0	0
		160	4	2	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
		320	3	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
		640	3	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
SC			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

X — Reciprocal of serum dilution
XX — Reciprocal of antigen dilution
AC — Antigen control
SC — Serum control

TABLE II

Immune-adherence test. Group A. Crude brain tissue antigens

SERA / ANTIGENS		X / XX	EEE 20	EEE 40	EEE 80	EEE 160	EEE 320	EEE 640	VEE 20	VEE 40	VEE 80	VEE 160	VEE 320	VEE 640	MAYARO 20	MAYARO 40	MAYARO 80	MAYARO 160	MAYARO 320	MAYARO 640	AURA 20	AURA 40	AURA 80	AURA 160	AURA 320	AURA 640	UNA 20	UNA 40	UNA 80	UNA 160	UNA 320	UNA 640	PIXUNA 20	PIXUNA 40	PIXUNA 80	PIXUNA 160	PIXUNA 320	PIXUNA 640	AC	TA
EEE		40	4	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		80	4	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		160	4	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		320	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VEE		40	0	0	0	0	0	0	4	4	4	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
		80	0	0	0	0	0	0	4	4	4	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
		160	0	0	0	0	0	0	4	4	4	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
		320	0	0	0	0	0	0	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		640	0	0	0	0	0	0	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAYARO		40	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		80	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		160	0	0	0	0	0	0	0	0	0	0	0	0	4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		320	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AURA		40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UNA		40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	0	0	0	0	0	0	0	0	0	0
		80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	0	0	0	0	0	0	0	0	0	0
		160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	0	0	0	0	0	0	0	0	0	0
		320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
		640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
PIXUNA		40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	0	0
		80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	3	0	0
		160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	2	1	0	0	0
		320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SC			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

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TABLE III

Immune-adherence test. Group A. Purified brain tissue antigens

SERA / ANTIGENS		EEE						VEE						MAYARO						AURA						UNA						PIXUNA						AC
		20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	
EEE	40	4	4	4	4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	80	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	160	4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VEE	40	0	0	0	0	0	0	4	4	4	4	3	0	4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0
	80	0	0	0	0	0	0	4	4	4	4	4	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0
	160	0	0	0	0	0	0	4	4	4	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
	320	0	0	0	0	0	0	4	4	3	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
	640	0	0	0	0	0	0	2	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
MAYARO	40	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	80	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	160	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	320	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	640	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AURA	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UNA	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	0	0	0	0	0	0	0	0	0
	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	0	0	0	0	0	0	0	0	0
	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	0	0	0	0	0	0	0	0	0
	320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	3	2	0	0	0	0	0	0	0	0	0
	640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	0	0	0	0	0	0	0	0	0	0
PIXUNA	40	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	3	0	0
	80	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	3	0	0
	160	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	3	0	0
	320	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	3	3	0	0
	640	0	0	0	0	0	0	T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	2	0	0	0	0
SC		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

TABLE IV

Immune-adherence test. Group B. Crude brain tissue antigens

SERA / ANTIGENS		YF						ILHEUS						BUSSUQUARA						SLE						AC
		20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	
YF	40	4	4	4	4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	80	4	4	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	160	4	4	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	320	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	640	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ILHEUS	40	4	0	0	0	0	0	0	4	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	80	4	0	0	0	0	0	0	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	160	4	0	0	0	0	0	0	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	320	4	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	640	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BUSSUQUARA	40	4	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	2	0	0	0	0	0	0	0
	80	4	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	3	0	0	0	0	0	0	0
	160	4	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	1	0	0	0	0	0	0	0
	320	4	0	0	0	0	0	0	0	0	0	0	0	0	4	4	1	0	0	0	0	0	0	0	0	0
	640	4	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0
SLE	40	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	1	0	0	0
	80	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0
	160	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0
	320	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
	640	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SC	TS	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

TABLE V

Immune-adherence test. Group B. Purified brain tissue antigens

ANTIGENS \ SERA		YF						ILHEUS						BUSSUQUARA						SLE						AC
		20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	
YF	40	4	2	3	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	80	4	4	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	160	4	4	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	320	4	2	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	640	4	2	3	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ILHEUS	40	4	0	0	0	0	0	0	3	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	80	4	0	0	0	0	0	0	3	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	160	4	0	0	0	0	0	0	3	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	320	4	0	0	0	0	0	0	2	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	640	4	0	0	0	0	0	0	2	3	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BUSSUQUARA	40	4	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	1	0	0	0	0	0	0	0
	80	4	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	2	0	0	0	0	0	0	0
	160	4	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	2	0	0	0	0	0	0	0
	320	4	0	0	0	0	0	0	0	0	0	0	0	0	3	4	4	4	3	0	0	0	0	0	0	0
	640	4	0	0	0	0	0	0	0	0	0	0	0	0	3	4	4	3	2	0	0	0	0	0	0	0
SLE	40	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	0	0	0
	80	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	0	0	0
	160	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	0	0	0
	320	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	0	0	0
	640	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	4	3	0	0	0
SC	TS	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

TABLE VI

Immune-adherence test. Group C. Crude liver tissue antigens

ANTIGENS \ SERA		MARITUBA						APEU						ORIBOCA						MURUTUCU						CARAPARU						ITAQUI						AC
		20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	
MARITUBA	40	4	4	4	4	4	2	4	4	4	4	2	2	4	4	3	2	0	0	4	1	0	0	0	0	3	2	0	0	0	0	4	4	3	0	0	0	0
	80	4	4	4	4	3	1	4	4	4	3	0	0	4	4	3	1	0	0	4	4	0	0	0	0	3	0	0	0	0	0	3	3	3	0	0	0	0
	160	4	4	4	3	2	0	4	3	3	2	0	0	3	3	2	0	0	0	4	3	0	0	0	0	3	0	0	0	0	0	3	0	0	0	0	0	0
	320	4	4	4	2	0	0	3	3	2	0	0	0	2	1	0	0	0	0	3	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0
	640	4	4	3	0	0	0	3	2	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
APEU	40	4	4	4	4	4	2	4	4	4	4	4	3	4	4	3	2	0	0	4	4	3	2	0	0	3	2	0	0	0	0	4	3	0	0	0	0	0
	80	4	4	4	4	4	0	4	4	4	4	4	2	4	4	3	1	0	0	4	4	3	0	0	0	3	1	0	0	0	0	3	2	0	0	0	0	0
	160	4	4	4	4	3	0	4	4	4	4	4	1	4	4	3	1	0	0	4	3	2	0	0	0	3	0	0	0	0	0	3	0	0	0	0	0	0
	320	4	4	3	2	1	0	4	4	3	2	1	0	4	2	0	0	0	0	4	1	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0
	640	4	3	2	0	0	0	4	3	2	1	0	0	2	2	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
ORIBOCA	40	4	4	3	2	0	0	4	4	4	2	0	0	4	4	4	4	3	1	4	4	4	4	3	1	4	3	0	0	0	0	4	3	0	0	0	0	0
	80	4	4	3	1	0	0	4	4	4	0	0	0	4	4	4	3	2	0	4	4	3	3	1	0	3	2	0	0	0	0	3	0	0	0	0	0	0
	160	4	4	3	1	0	0	4	4	3	0	0	0	4	4	4	2	0	0	4	4	3	2	0	0	3	0	0	0	0	0	2	0	0	0	0	0	0
	320	3	2	0	0	0	0	4	3	0	0	0	0	4	4	3	0	0	0	4	3	2	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0
	640	1	0	0	0	0	0	2	1	0	0	0	0	4	2	0	0	0	0	4	3	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
MURUTUCU	40	4	4	3	1	0	0	4	4	4	0	0	0	4	4	4	4	3	0	4	4	4	4	0	0	4	2	0	0	0	0	4	0	0	0	0	0	0
	80	4	3	2	0	0	0	4	4	4	0	0	0	4	4	4	4	3	0	4	4	4	4	0	0	4	0	0	0	0	0	4	0	0	0	0	0	0
	160	4	3	1	0	0	0	4	4	4	0	0	0	4	4	4	3	3	0	4	4	4	4	0	0	4	0	0	0	0	0	4	0	0	0	0	0	0
	320	3	1	0	0	0	0	4	3	2	0	0	0	4	4	3	3	0	0	4	4	3	2	0	0	3	0	0	0	0	0	3	0	0	0	0	0	0
	640	0	0	0	0	0	0	3	1	0	0	0	0	4	3	0	0	0	0	4	4	2	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0
CARAPARU	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	3	0	0	4	4	4	3	3	1	0
	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	2	0	0	4	4	4	4	1	0	0
	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	2	3	3	2	0	0	0
	320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0
	640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ITAQUI	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	0	0	4	4	4	3	3	1	0
	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	2	0	0	4	4	3	3	0	0	0
	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	2	0	0	0	4	3	3	0	0	0	0
	320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	2	0	0	0	4	4	3	0	0	0	0
	640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	1	0	0	0	4	3	3	0	0	0	0
SC		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

TABLE VII

Immune-adherence test. Guamá complex

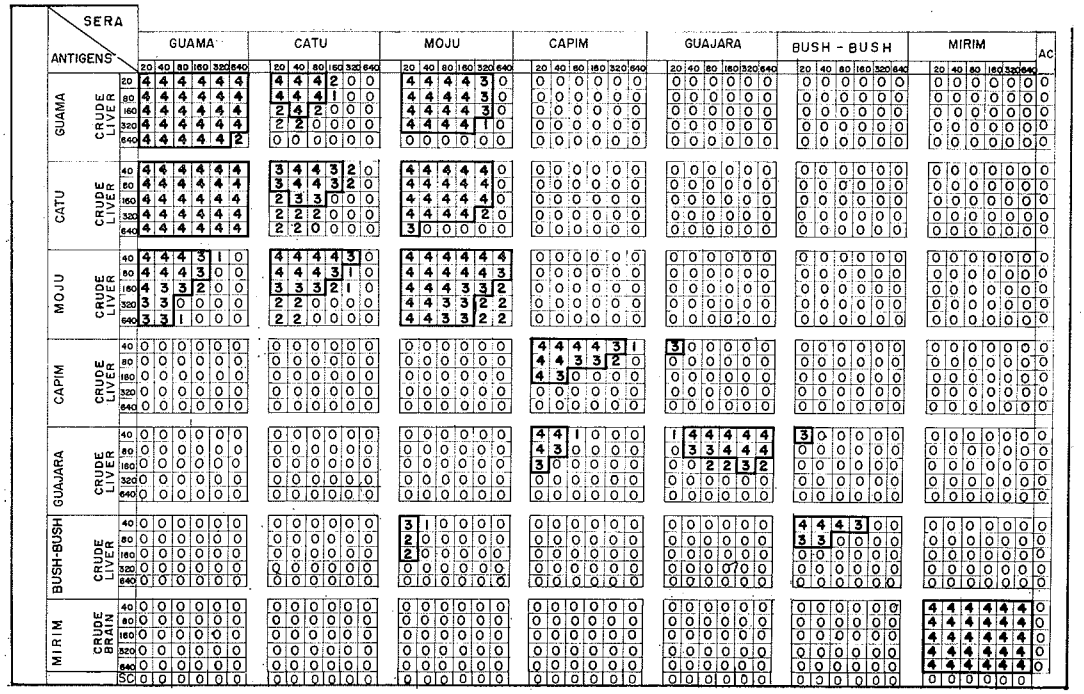

TABLE VIII

Complement fixation test. Group C. Crude liver tissue antigens

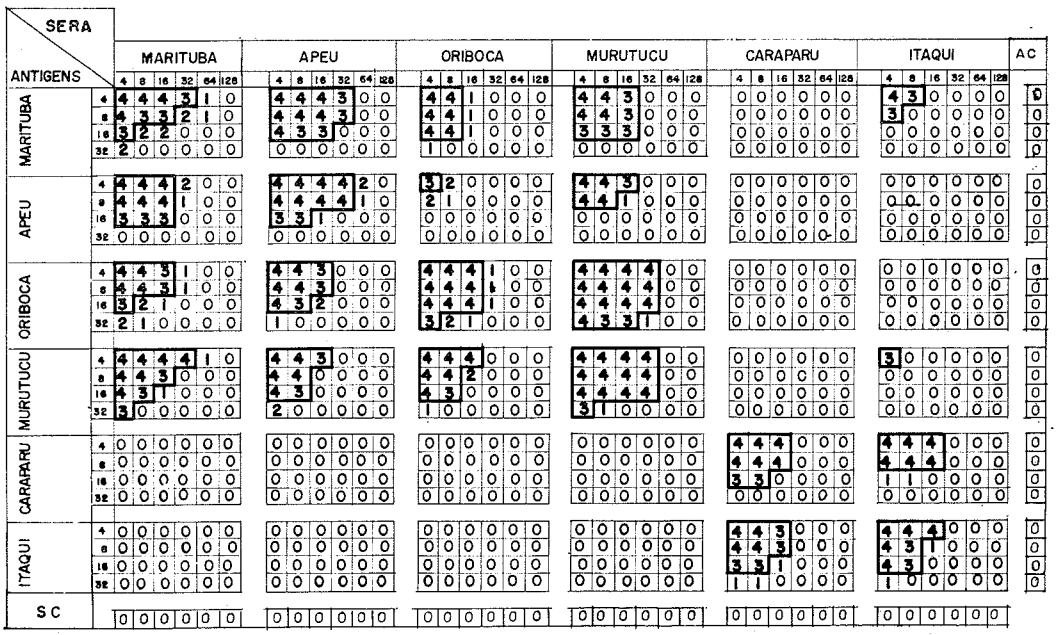

TABLE IX

Immune-adherence test. Group A bivalent and trivalent sera. Crude brain tissue antigens

SERA / ANTIGENS	EEE UNA						EEE · VEE						EEE AURA						EEE · VEE MAYARO						AC
	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	
EEE 40	4	4	4	3	0	0	4	4	4	3	0	0	4	4	4	3	0	0	4	4	4	4	0	0	0
EEE 80	4	4	4	1	0	0	4	4	4	2	0	0	4	4	4	3	0	0	4	4	4	3	0	0	0
EEE 160	4	4	3	0	0	0	4	4	3	0	0	0	4	4	4	2	0	0	4	4	4	2	0	0	0
EEE 320	4	3	2	0	0	0	4	3	3	0	0	0	4	4	3	0	0	0	4	3	0	0	0	0	0
EEE 640	4	3	1	0	0	0	4	3	3	0	0	0	4	3	3	0	0	0	4	3	0	0	0	0	0
VEE 40	4	4	1	0	0	0	4	4	4	4	3	0	3	3	0	0	0	0	4	4	4	4	4	1	0
VEE 80	4	2	0	0	0	0	4	4	4	4	1	0	3	2	0	0	0	0	4	4	4	4	3	0	0
VEE 160	4	0	0	0	0	0	4	4	4	3	0	0	3	0	0	0	0	0	4	4	4	4	3	0	0
VEE 320	1	0	0	0	0	0	4	4	3	0	0	0	0	0	0	0	0	0	4	4	4	4	2	0	0
VEE 640	0	0	0	0	0	0	4	4	2	0	0	0	0	0	0	0	0	0	4	4	4	3	0	0	0
MAYARO 40	4	1	0	0	0	0	4	3	0	0	0	0	4	3	0	0	0	0	4	4	4	3	0	0	0
MAYARO 80	4	0	0	0	0	0	4	0	0	0	0	0	3	2	0	0	0	0	4	4	4	3	0	0	0
MAYARO 160	4	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	4	4	3	2	0	0	0
MAYARO 320	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	2	0	0	0	0
MAYARO 640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	0	0	0	0	0
AURA 40	4	4	2	0	0	0	3	3	0	0	0	0	4	4	4	4	0	0	4	3	0	0	0	0	0
AURA 80	4	3	0	0	0	0	3	1	0	0	0	0	4	4	4	3	0	0	3	2	0	0	0	0	0
AURA 160	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	3	0	0	3	0	0	0	0	0	0
AURA 320	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	0	0	0	0	0	0	0	0	0	0
AURA 640	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0	0
UNA 40	4	4	4	3	0	0	3	2	0	0	0	0	4	4	4	3	0	0	4	3	0	0	0	0	0
UNA 80	4	4	3	0	0	0	3	0	0	0	0	0	4	4	3	3	0	0	3	1	0	0	0	0	0
UNA 160	4	4	3	0	0	0	2	0	0	0	0	0	4	4	3	0	0	0	0	0	0	0	0	0	0
UNA 320	4	4	3	0	0	0	0	0	0	0	0	0	4	4	3	2	0	0	0	0	0	0	0	0	0
UNA 640	4	3	0	0	0	0	0	0	0	0	0	0	4	3	1	0	0	0	0	0	0	0	0	0	0
PIXUNA	3	2	0	0	0	0	4	3	0	0	0	0	3	2	0	0	0	0	3	3	0	0	0	0	0
	3	0	0	0	0	0	3	0	0	0	0	0	3	0	0	0	0	0	3	2	0	0	0	0	0
	2	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

RESULTS

Comparison of mouse brain, liver, and serum as sources of IA antigen — Guamá virus was used to study the localization of IA antigen in different tissues of the baby mouse. Table I shows that IA antigen was produced in optimal titers in liver tissue and in lower titers in brain and serum. Previous studies [11] at the Belém Virus Laboratory had shown that the CF antigen with the AN 277 strain of Guamá virus was also produced optimally in the liver, whereas hemagglutinin appeared in higher titer in the serum. These results led to the employment as sources of IA antigen for other viruses, that tissue known to yield optimal CF antigen titers.

Comparative study of the IA reaction with crude and purified antigens — Crude and purified mouse brain antigens for viruses in groups A and B were compared. Results are presented in Tables II, III, IV and V. Reactions between antigens and hyperimmune sera in each group showed no

superiority of one type of antigen over the other. Therefore crude antigens were adopted for subsequent experiments.

Study of the sensitivity and specificity of the IA test — IA testing was satisfactorily carried out with viruses of groups C and Guamá, complex Capim, and Mirim virus (Tables VI and VII) in addition to those of groups A and B (Tables II and IV). The IA test was more sensitive than the CF test, higher IA titers being obtained than those usually obtained in CF reactions.

The specificity of the IA test was good for groups A and B, but not for group Guamá. This lack of specificity parallels that described for CF testing [14] with the group Guamá viruses. Similarly for the Capim complex and Mirim virus, the IA reactions

TABLE X

Complement fixation test. Group A bivalent and trivalent sera. Crude brain tissue antigens

SERA / ANTIGENS		EEE UNA				EEE · VEE				EEE AURA UNA				EEE · VEE MAYARO				AC
		2	4	8	16	2	4	8	16	2	4	8	16	2	4	8	16	
EEE	4	4	4	4	4	4	3	2	0	4	4	4	3	4	3	2	0	0
	8	4	4	4	4	3	3	2	0	4	4	4	3	4	3	2	0	0
	16	4	4	4	4	3	3	2	0	4	4	4	3	3	2	2	0	0
	32	4	3	2	2	3	1	0	0	3	3	2	0	3	2	1	0	0
VEE	4	0	0	0	0	4	4	4	4	0	0	0	0	4	4	4	4	0
	8	0	0	0	0	4	4	4	4	0	0	0	0	4	4	4	4	0
	16	0	0	0	0	4	4	4	4	0	0	0	0	4	4	4	4	0
	32	0	0	0	0	4	4	3	2	0	0	0	0	3	2	2	1	0
MAYARO	4	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	0
	8	0	0	0	0	0	0	0	0	0	0	0	0	4	4	3	0	0
	16	0	0	0	0	0	0	0	0	0	0	0	0	2	3	1	0	0
	32	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
AURA	4	0	0	0	0	0	0	0	0	4	4	2	1	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	4	4	2	0	0	0	0	0	0
	16	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0
UNA	4	4	4	4	4	0	0	0	0	4	4	4	2	0	0	0	0	0
	8	4	4	4	4	0	0	0	0	4	4	4	2	0	0	0	0	0
	16	4	4	4	4	0	0	0	0	4	4	4	0	0	0	0	0	0
	32	3	3	2	2	0	0	0	0	4	4	3	1	0	0	0	0	0
PIXUNA	4	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0
	8	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S C		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

TABLE XI

Immune-adherence test. Group B bivalent sera. Crude brain tissue antigens

SERA / ANTIGENS	ILHEUS S L E						YF ILHEUS						BUSSUQUARA YF						BUSSUQUARA ILHEUS						YF S L E						BUSSUQUARA S L E						A C
	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	20	40	80	160	320	640	TA
YF 40	4	3	0	0	0	0	4	4	4	3	0	0	4	4	4	4	3	0	4	3	0	0	0	0	4	4	4	4	3	0	3	3	0	0	0	0	0
YF 80	3	0	0	0	0	0	4	4	4	3	0	0	4	4	4	3	3	0	4	3	0	0	0	0	4	4	4	3	0	0	3	1	0	0	0	0	0
YF 160	0	0	0	0	0	0	4	4	3	2	0	0	4	4	4	3	0	0	3	0	0	0	0	0	4	4	4	2	0	0	3	0	0	0	0	0	0
YF 320	0	0	0	0	0	0	4	3	2	1	0	0	4	4	3	2	0	0	0	0	0	0	0	0	4	4	3	1	0	0	0	0	0	0	0	0	0
YF 640	0	0	0	0	0	0	3	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	4	3	1	0	0	0	0	0	0	0	0	0	0
ILHEUS 40	4	4	4	4	3	0	4	4	4	4	4	3	4	3	1	0	0	0	4	4	4	4	3	0	4	3	1	0	0	0	4	2	0	0	0	0	0
ILHEUS 80	4	4	4	3	0	0	4	4	4	4	3	0	4	3	0	0	0	0	4	4	3	3	0	0	3	2	0	0	0	0	3	0	0	0	0	0	0
ILHEUS 160	4	4	4	3	0	0	4	4	3	3	0	0	3	2	0	0	0	0	4	4	3	1	0	0	3	0	0	0	0	0	2	0	0	0	0	0	0
ILHEUS 320	4	4	3	0	0	0	4	3	2	0	0	0	0	0	0	0	0	0	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ILHEUS 640	4	3	1	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0	4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BUSSUQUARA 40	4	4	3	0	0	0	4	3	0	0	0	0	4	4	3	2	0	0	4	4	3	1	0	0	3	3	0	0	0	0	4	4	4	3	0	0	0
BUSSUQUARA 80	4	3	0	0	0	0	3	0	0	0	0	0	4	3	1	0	0	0	4	3	2	0	0	0	3	2	0	0	0	0	4	4	3	3	0	0	0
BUSSUQUARA 160	3	3	0	0	0	0	2	0	0	0	0	0	3	3	2	1	0	0	3	3	1	0	0	0	2	0	0	0	0	0	4	3	3	0	0	0	0
BUSSUQUARA 320	2	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	4	3	1	0	0	0	0
BUSSUQUARA 640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
S L E 40	4	4	3	2	0	0	4	4	2	0	0	0	3	3	0	0	0	0	4	4	3	1	0	0	4	4	3	0	0	0	4	4	4	3	0	0	0
S L E 80	4	4	3	0	0	0	4	2	0	0	0	0	3	2	0	0	0	0	4	3	3	0	0	0	4	4	3	0	0	0	4	4	4	1	0	0	0
S L E 160	4	4	3	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	4	4	3	0	0	0	4	4	3	0	0	0	0
S L E 320	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	4	3	0	0	0	0	4	3	2	0	0	0	0
S L E 640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	3	1	0	0	0	0	0
S C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

TABLE XII

Complement fixation test. Group B bivalent sera. Crude brain tissue antigens

SERA / ANTIGENS	ILHÉUS S L E				YF ILHEUS				YF BUSSUQUARA				BUSSUQUARA ILHÉUS				YF SLE				BUSSUQUARA S L E				AC
	2	4	8	16	2	4	8	16	2	4	8	16	2	4	8	16	2	4	8	16	2	4	8	16	
YF 4	1	0	0	0	4	3	0	0	4	4	4	2	0	0	0	0	4	4	4	0	0	0	0	0	0
YF 8	0	0	0	0	4	3	0	0	4	4	4	3	0	0	0	0	4	4	4	0	0	0	0	0	0
YF 16	0	0	0	0	4	3	0	0	4	4	4	3	0	0	0	0	4	4	4	2	0	0	0	0	0
YF 32	0	0	0	0	3	1	0	0	3	2	1	0	0	0	0	0	4	3	0	0	0	0	0	0	0
ILHEUS 4	4	4	3	3	4	4	4	0	0	0	0	0	4	4	4	1	0	0	0	0	0	0	0	0	0
ILHEUS 8	4	4	3	1	4	4	4	1	0	0	0	0	4	4	4	2	0	0	0	0	0	0	0	0	0
ILHEUS 16	4	4	3	0	4	4	4	3	0	0	0	0	4	4	4	2	0	0	0	0	0	0	0	0	0
ILHEUS 32	4	4	3	0	4	4	4	2	0	0	0	0	4	4	4	1	0	0	0	0	0	0	0	0	0
BUSSUQUARA 4	4	3	0	0	2	0	0	0	3	0	0	0	3	0	0	0	0	0	0	0	2	0	0	0	0
BUSSUQUARA 8	3	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BUSSUQUARA 16	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BUSSUQUARA 32	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S L E 4	4	3	1	0	3	1	0	0	0	0	0	0	4	3	0	0	4	3	0	0	4	1	0	0	0
S L E 8	4	3	1	0	1	0	0	0	0	0	0	0	4	3	0	0	4	3	0	0	4	1	0	0	0
S L E 16	4	3	1	0	0	0	0	0	0	0	0	0	1	0	0	0	4	3	0	0	4	3	1	0	0
S L E 32	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	0	4	3	1	0	0
S C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

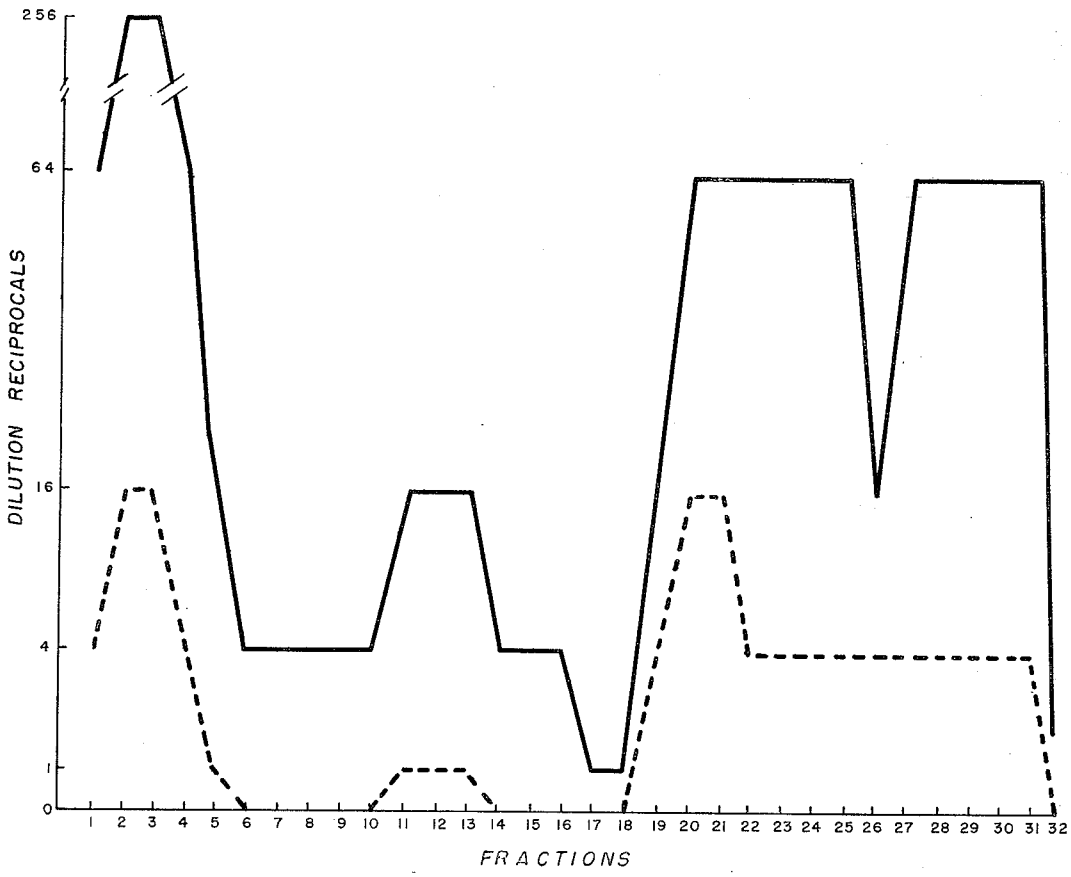

Fig. 1 — Detection of IA and CF antigens of Guamá virus in DEAE-cellulose column fractionated material

——— IA test
..... CF test

are equivalent in specificity to those found by CF test [11].

In group C, three pairs of related viruses were noted by IA test (Table VI). These pairs of viruses coincide with those previously found by SHOPE & CAUSEY [12] in CF tests. Because of the large number of cross-reactions observed, CF testing using the same sera and antigens was carried out. The results (Table VIII) show a low degree of specificity of the sera employed, and an equivalent specificity of the IA and CF tests.

Bivalent and trivalent sera for group A and bivalent sera for group B were tested against the same antigens in CF and IA. Results in Table IX through XII indicate that both tests were adequate for identification of specific antibody. Heterologous, group-reacting antibody was detected to some degree by both tests, and was specially marked in serum from animals immunized with Bussuquara virus.

IA and CF testing of Guamá antigen fractionated on a DEAE-cellulose column — A Guamá virus preparation obtained from liver tissue of infected newborn mice, was fractionated on a DEAE-cellulose column. The fractions were tested for potency of the IA and CF antigens. In addition, all fractions were infective in newborn mice. Control NaCl fractions corresponding to the first, sixteenth, and thirty-second fractions were made, no death being observed.

As shown in Fig. 1, the presence of antigen in all fractions was demonstrated by

IA but not in all instances by CF tests. Furthermore, the presence of complement fixing antigen always coincided with high titers of the immune-adherence antigen; when the IA antigen exhibited low titers, the presence of CF antigen could not be demonstrated. There is no evidence for separation of the IA and CF antigens.

DISCUSSION

The IA test could be employed with all arboviruses studied. The IA antigen is very similar to the CF antigen in its localization in mouse tissue, its specificity within several different groups of arboviruses, and in its behavior when fractionated on a DEAE-cellulose column. The IA test has a greater sensitivity than the CF test under the conditions of this study. Titers ten-fold higher than those of CF, were frequently obtained by IA tests employing the same sera and antigens. Besides, the presence of viral antigens could be detected in the same material where CF tests provided negative results. The simplicity and the rapidity of the reaction recommend the IA test for more intensive trial in the study of the arthropod-borne viruses.

RESUMO

O emprêgo da reação de imuno-aderência no estudo de alguns arbovírus brasileiros

A prova de imuno-aderência foi utilizada no estudo sorológico de arbovírus dos grupos A, B, C, Guamá e complexo Capim. A comparação entre as reações de imuno-aderência e fixação do complemento revelaram especificidade semelhante, aparecendo sempre a imuno-aderência com maior sensibilidade. Antígeno de imuno-aderência para o vírus Guamá foi encontrado em maiores títulos no fígado, que no cérebro e sôro de camundongos recém-nascidos infetados. As atividades para imuno-aderência e fixação do complemento não puderam ser separadas em coluna de DEAE celulose.

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