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Arbovirus neutralization tests with Peruvian sera in Vero cell cultures

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Selected human sera from Peru, previously examined by the haemagglutination–inhibition (HI) test with a number of arboviruses, were reexamined by neutralization tests carried out in Vero cell cultures. Results confirmed and extended the HI findings, indicating that the antibodies detected were evoked by Eastern equine encephalitis, Mayaro, Venezuelan equine encephalitis, Ilheus, St Louis encephalitis, yellow fever, Caraparu, and Guaroa viruses.

Over 1 000 serum specimens collected from residents of eastern Peru during March–July 1965 were subsequently examined with 27 different arbovirus antigens in haemagglutination-inhibition (HI) tests carried out at this laboratory by three of us (J.M., W.F., and J.C.). The results (to be reported) indicated widespread experience by the population surveyed with the following arboviruses (or closely related agents): Eastern equine encephalitis (EEE), Mayaro, Venezuelan equine encephalitis (VEE), Ilheus, St Louis encephalitis (SLE), yellow fever (YF), Caraparu, Murutucu, Guaroa, and Maguari. Occasional sera reacted with antigen for Bussuquara virus or for bat salivary gland virus.

These findings provided the basis for selecting sera for reexamination by neutralization (N) tests carried out in Vero cell cultures with potentiating accessory factor (Morgan, 1945; Whitman, 1947; Taylor et al., 1955). This paper reports the N-test results obtained and compares them with the HI-test data.

MATERIALS AND METHODS

Sera

The donors of the survey sera were apparently healthy males and females, 5–75 years of age, resident in the half of Peru lying east of the eastern foothills of the Andes. After collection the sera were stored in a freezer at −20°C. For the N tests, the specimens were inactivated at 56°C for 30 min immediately before use.

Virus strains

The following viruses were used: EEE (TenBroeck), Mayaro (Tr 4675), Una (BeAr 13136), VEE (Trinidad Donkey No. 1), Mucambo (BeAn 8), Pixuna (BeAr 35645), Ilheus (original), SLE (BeAr 23379), YF (Asibi), Caraparu (BeAn 3994), and Guaroa (CoH 35211). Virus stocks, representing the lyophilized infected fluid phase of either the first or the second passage of each virus in Vero cell cultures, were held in 1-ml ampoules in a freezer at −20°C until used. For tests, two ampoules of the given virus were rehydrated, pooled, and held at room temperature for 20 min prior to dilution.

Cell cultures

Stationary tube cultures of Vero cell monolayers in the fluid phase were used according to procedures already described (Buckley & Casals, 1970).

Accessory factor

A supply of normal rhesus monkey serum,[1] known to be free of virus antibodies and of virucidal and cytotoxic substances, was kept in a freezer at −65°C in screw-cap tubes (2–5 ml).

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Diluent

The diluent consisted of 0.75% bovine plasma albumin (Armour fraction V) in 0.05 M phosphate-buffered saline, pH 7.2.

Virus titrations

Two ampoules of the given virus were rehydrated, pooled, and held at room temperature for 20 min before being prepared as serial 10-fold dilutions with diluent. Each dilution was inoculated into three replicate Vero cell cultures, which were examined at intervals for the development of cytopathic effect (CPE). Virus titres were calculated by the method of Reed & Muench (1938) and are expressed as cytopathic dose$_{50}$ (CPD_{50}) index (Haldane, 1960) per millilitre.

N tests

In the qualitative N test, approximately 200 CPD_{50} of virus was mixed in equal volumes with an undiluted, inactivated survey serum. To this serum–virus mixture was added an equal amount of accessory factor (AF, normal rhesus monkey serum), the latter thus making up 50% of the total mixture. After incubation for 1 hour at 37°C, the mixture was inoculated into two Vero cell cultures, 0.1 ml per tube. With each qualitative test, virus titrations were carried out on the day of test. Hyperimmune mouse sera for the respective viruses, as well as human sera without antibodies, served as additional controls. Tests were terminated when the expected end-point of the virus titration was obtained.

The quantitative N test with AF was carried out as described above, with certain modifications. (1) Each survey serum was diluted serially twofold from 1 : 2 to 1 : 1024. (2) Each control hyperimmune mouse serum was also diluted 1 : 2 to 1 : 1024, and each control human serum was diluted 1 : 2. (3) As an additional control, the virus dose used, 200 CPD_{50}, was mixed in equal parts with diluent, to which mixture was then added an equal part of AF (representing 50% of the total mixture); after incubation for 1 hour at 37°C, the mixture was immediately diluted serially 10-fold and each dilution was inoculated into 6–12 Vero cell cultures. The CPD_{50} titres obtained from these titrations accurately indicated the actual CPD_{50} used in the quantitative N test with AF.

In addition, quantitative N tests were carried out in which AF was replaced throughout by inactivated normal rhesus monkey serum. Inactivation was performed by heating serum in 1-ml amounts for 30 min at 56°C immediately before the tests.

Table 1. Results obtained with selected Peruvian sera by qualitative neutralization tests in Vero cell cultures with accessory factor, compared with HI test survey results

Virus		Sera		
Sero-group	Name	No. tested	No. protective in N test	No. positive in HI test
A	EEE	11	8	8
	Mayaro	100	68	70
	Mucambo	100	53	58
	Pixuna	100	13	49
	Una	84	54	41
	VEE	100	64	65
B	Ilheus	89	58	57
	SLE	8	7	8
	YF	84	52	54
C	Caraparu	100	65	68
Bunyamwera	Guaroa	100	68	70

RESULTS

Qualitative N test

In these tests, a serum was considered " protective " if the serum–virus–AF mixture failed to induce CPE in the two Vero cell cultures inoculated. As shown in Table 1, there was good correlation between the results of this test and the HI results with all viruses except Una and Pixuna. With Una virus, 54 of the 84 sera tested were protective, whereas only 41 of the 84 were HI-positive. With Pixuna, the respective ratios were 13/100 and 49/100.

Quantitative N test

In these tests, the serum neutralizing titre was expressed as the highest serum dilution giving protection in one (50%) of the two Vero cell cultures inoculated.

Table 2 shows the results obtained when 15 survey sera, characterized by HI pattern as " anti-Mayaro ", were tested against Mayaro and Una viruses in tests performed with and without AF. In the presence of AF, each serum neutralized Mayaro virus to a higher titre than it did Una virus, the data thus confirming the HI findings. In the absence of AF, the N test titres were exceedingly low and it was impossible to say which virus was responsible for the antibody detected. Since the titres obtained with the remaining sera in the absence of AF were

Table 2. Quantitative neutralization tests, carried out with and without accessory factor, with Mayaro and Una viruses and 15 Peruvian sera characterized by HI pattern as " anti-Mayaro " [a]

Serum no.	N test titre			HI titre	
	Accessory factor	Mayaro	Una	Mayaro	Una
258	Yes	256	<2	20	10
	No	2	<2		
552	Yes	⩾32	8	20	10
	No	4	6		
821	Yes	⩾32	12	20	10
	No	2	4		
647	Yes	512	6	40	10
	No	<2	3		
692	Yes	384	3	40	10
	No	<2	<2		
194	Yes	1 024	8	80	20
	No	3	2		
130	Yes	384	16	160	20
	No	2	8		
183	Yes	768	8	160	20
	No	2	4		
241	Yes	768	24	160	20
	No	2	4		
275	Yes	⩾1 024	6	160	10
	No	2	6		
266	Yes	⩾1 024	16	640	80
	No	3	6		
925	Yes	768	32	640	80
	No	3	2		
213	Yes	⩾1 024	128	1 280	80
	No	32	16		
373	Yes	⩾1 024	32	1 280	80
	No	6	3		
786	Yes	192	32	1 280	40
	No	2	8		

[a] 50 CPD_{50} of Mayaro virus and 40 CPD_{50} of Una virus were used in test (final dilution). All titres are expressed as the reciprocal of the serum dilution.

Table 3. Neutralization tests, using accessory factor, carried out with 22 Peruvian sera characterized by HI pattern as " anti-EEE " or " anti-VEE "

HI titre [a] range	8 " anti-EEE " sera: N test titres with	14 " anti-VEE " sera: N test titres with		
	EEE	VEE	Mucambo	Pixuna
20–40	32–64	12–48	<2–8	<2–12
80–160	128–256	64–384	3–24	6–64
⩾160		⩾1 024	48–96	16–48

[a] HI titres with EEE and VEE viruses, respectively. All titres are expressed as the reciprocal of the serum dilution.

also low, on the whole, the subsequent tables report only the data for N tests performed with AF.

As shown in Table 3, for 14 sera characterized by HI pattern as " anti-EEE ", N test titres with EEE virus were consistently higher than the HI titres. For 14 sera characterized as " anti-VEE ", cross-N test results also confirmed the survey findings and indicated that VEE, rather than Mucambo or Pixuna virus, was responsible for the antibody detected.

Table 4 summarizes the results of cross-N tests with Ilheus, SLE, and YF viruses and 36 sera characterized by HI pattern as either " anti-Ilheus ", " anti-SLE ", or " anti-YF ". With very few exceptions, the data again confirmed the HI findings.

For 15 survey sera characterized as " anti-Caraparu ", N test titres with Caraparu virus perfectly matched HI titres (Table 5). N test results with 15 " anti-Guaroa " sera and Guaroa virus likewise confirmed the HI results (Table 6), although in this instance some discrepancies in titre were observed.

Table 4. Neutralization tests, using accessory factor, carried out with 36 Peruvian sera characterized by HI pattern as " anti-Ilheus ", " anti-SLE ", or " anti-yellow fever "

HI titre [a] range	13 " anti-Ilheus " sera: N test titres with			8 " anti-SLE " sera: N test titres with			15 " anti-YF " sera: N test titres with		
	Ilheus	SLE	YF	Ilheus	SLE	YF	Ilheus	SLE	YF
20–40	32–64	<2	<2–16	<2–4	24–48	2–3	<2–3	<2–6	4–64
80–160	32–128	<2	4–48	<2–3	48–128	8–16	2–6	<2–12	128–256
160 +	16–768	2–64 +	8–512	<2–2	<2–24	4–16	<2–192	<2–64	96–192

[a] HI titres with, respectively, Ilheus virus (" anti-Ilheus " sera), SLE virus (" anti-SLE " sera), and YF virus (" anti-YF " sera). All titres are expressed as the reciprocal of the serum dilution.

Table 5. Neutralization tests, using accessory factor, carried out with Caraparu virus and 15 Peruvian sera characterized by HI pattern as "anti-Caraparu" [a]

Serum no.	N test titre	HI titre
965	<2	10
555	8	10
122	16	10
52	32 +	20
524	32 +	20
957	32 +	20
513	64	40
106	64 +	40
173	64 +	40
635	128	80
1024	128	80
817	128	160
512	256 +	160
841	256 +	160
445	512 +	320

[a] All titres are expressed as the reciprocal of the serum dilution.

Table 6. Neutralization tests, using accessory factor, carried out with Guaroa virus and 15 Peruvian sera characterized by HI pattern as "anti-Guaroa" [a]

Serum no.	N test titre	HI titre
290	8	20
678	48	20
810	32	40
142	128	40
203	192	40
702	384	40
353	64	80
622	256	80
734	32	160
417	192	160
160	192	320
887	512	320
826	384	640
125	1 024 +	640
947	128	1 280

[a] All titres are expressed as the reciprocal of the serum dilution.

DISCUSSION

The Vero cell monolayer (in fluid phase) technique described in this paper may be used as a simple qualitative N test or as a more cumbersome, but also more relevant, quantitative N test to confirm and extend serological patterns established in extensive HI test surveys. The comparative data presented in Tables 2–6 show that, for EEE, Mayaro, VEE, Ilheus, SLE, YF, Caraparu, and Guaroa viruses, the quantitative N test with AF is as sensitive as the HI test.

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RÉSUMÉ

ÉPREUVES DE NEUTRALISATION D'ARBOVIRUS PRATIQUÉES À L'AIDE DE SÉRUMS DE PÉRUVIENS EN CULTURES CELLULAIRES VERO

Plus de 1000 sérums prélevés en 1965 chez des habitants de l'est du Pérou avaient été examinés en épreuves d'inhibition de l'hémagglutination (IH) en présence d'une série d'arbovirus. On avait relevé un fort taux de positivité à l'égard des virus: encéphalomyélite équine de l'Est, Mayaro, encéphalomyélite équine du Venezuela, Ilheus, encéphalite de St Louis, fièvre jaune, Caraparu, Murutucu, Guaroa et Maguari.

On a choisi un certain nombre de ces sérums et on les a réexaminés en épreuves de neutralisation (N) qualitatives et quantitatives pratiquées en cultures cellulaires Vero avec addition de facteur accessoire (sérum de singe rhésus). En épreuves qualitatives, on a noté une corrélation satisfaisante entre les résultats des épreuves IH et N, sauf en ce qui concerne les virus Una et Pixuna. Le test quantitatif s'est révélé aussi sensible que le test IH et a permis de confirmer les données fournies par ce dernier.

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