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HEMAGGLUTININ PRODUCTION AND INFECTIVITY PATTERNS IN ADULT HAMSTERS INOCULATED WITH GROUP C AND OTHER NEW WORLD ARBOVIRUSES*

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ABSTRACT: Most group C arboviruses were found to induce illness and death in adult hamsters. Sick animals had high viremia as determined by the presence of serum hemagglutinins. These agglutinins had specificity comparable to that of hemagglutinins from suckling-mouse serum. Thus the adult hamster was a more economical and convenient source of virus antigen than the infant mouse. Although serum hemagglutinins were not demonstrated, subcutaneous inoculation of hamsters caused lethal infection by Eastern, Western, and Venezuelan equine encephalomyelitis viruses, as well as Chagres virus, a member of the phlebotomus-fever group, suggesting that this animal may be a valuable sentinel animal for field studies of these agents.

Group C arboviruses were first isolated in the Amazon region of Brazil and have been encountered frequently in man and animals during the last decade.[1-8] Other strains have been reported from Trinidad[4-6] and Panamá.[13] Recently, Scherer and associates have isolated many strains of group C viruses in México.[7] Similar agents have been detected in the Florida Everglades by workers at the National Communicable Disease Center, Atlanta, Georgia.[9] Patois and Zegla viruses from Panamá, previously classified as group C,[10] have recently been separated into the "Patois group" which, together with group C and other related groups, is now included as a member of the Bunyamwera "super-group."[10,11,12]

Hemagglutinins produced by group C viruses are widely used in identification of viruses, as their specificity has been demonstrated to be comparable to that seen in neutralization tests.[13] The hemagglutination-inhibition (HI) technique is also valuable in serologic surveys for antibodies to the 11 presently known serotypes. These can be subdivided into at least five pairs: Apéu-Caraparú, Marituba-Murutucu, Itaqui-Oriboca, Madrid-Ossa, and Nepuyo-Gumbo Limbo. Presumably either one of the antigens in each pair, together with the unpaired antigen for Restan virus, could be incorporated in HI screening tests for group C arbovirus antibody surveys. Two viruses in the Patois group, Patois and Zegla, also form a serologic pair by HI tests, as demonstrated for group C agents.

A major problem, however, has been that hemagglutinating (HA) antigen for members of these groups frequently must be prepared from serum of infected suckling mice. Such a procedure gives a low yield and, in many cases, poor titers. Efforts to prepare group C HA antigens in cell cultures have met with little success.[14] We here describe preparation of HA antigens for group C and Patois viruses from the serum of adult hamsters, as well as preliminary data concerning the response of this animal to infection by 22 other arboviruses.

MATERIALS AND METHODS

Virus strains

Group C arboviruses that were used in this study were kindly supplied by different laboratories. Apéu (BeAn 848), Caraparú (BeAn 3994), Itaqui (BeAn 12752), Marituba (BeAn 151), Murutucu (BeAn 974), Nepuyo (BeAn 10709), and Oriboca (BeAn 17) viruses originally isolated from Belém, Brazil, were received from the Rockefeller Foundation Virus Laboratories in New York, in 1963. The Caraparú-like (TRVL 34053-1) and Restan (TRVL 51144) viruses were isolated from Trinidad. The prototype strains of Madrid (BT 4075) and Ossa (BT 1820) were recovered from Panamá. A strain of Nepuyo (63U11) originated in México.

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and a recently isolated strain of group C virus from Florida, Gumbo Limbo (Fe 371H), were also included in this study. In addition, two Patois group viruses from Panamá (Patois and Zegla), and a Mexican strain of Patois (63A49)[35] were studied.

Arboviruses other than those mentioned above were standard strains used at the Gorgas Memorial Laboratory for serologic work. They were originally obtained from various laboratories, as follows: a) The Rockefeller Foundation Virus Laboratories: Mucambo (BeAn 8), Pixuna (BeAr 35645), Mayaro (BeAr 20290), Bussuquara (BeAn 4116), Guamá (BeAn 277), and Catú (H 151) isolated from Brazil, and Bimiti (TRVL 8362) and Cocal (TRVL 40233), isolated from Trinidad; b) The National Communicable Disease Center in Atlanta: Western equine encephalomyelitis (Fleming strain), Cache Valley (6V 633), and California encephalitis (BFS 283) isolated from North America; c) The Middle America Research Unit, Canal Zone: Una (BT 1495), Guaroa (BT 1122), Wyeomyia (BT 219), Chagres (JW 10), Melao (BT 1113), Changuinola (BT 104), and VSV-Indiana (BT 78) isolated from Panamá; d) The Government of Panamá's Veterinary Laboratory: Eastern equine encephalomyelitis (strain #3478) from Panamá. In addition, Venezuelan equine encephalomyelitis (strain F 322), St. Louis encephalitis (strain BV 7), and Ilhéus (BT 3875), isolated at the Gorgas Memorial Laboratory, were used in the study.

Virus stocks were prepared as 20% clarified brain suspensions in phosphate saline solution containing 0.75% bovine albumin, and were stored at −70°C. Animals were inoculated with a 10^{-2} dilution, giving an infectious dose of 10^{3} to 10^{6} infant mouse LD_{50} for groups C and Patois viruses, and $10^{4.5}$ to 10^{8} infant mouse LD_{50} for other arboviruses.

Antiserum Samples

Samples of antiserum were prepared in adult mice by two intraperitoneal (ip) inoculations, 10 days apart, of infant-mouse liver virus. When viruses were lethal for adult mice, the first injection was made with formalin-inactivated virus; serum was obtained 10 days after the second injection. Virus titrations were done by intracerebral (ic) inoculation of decimal dilution into infant mice. Endpoints were calculated by the Reed-Muench method.[33]

Antigens and Serologic Tests

Serum for HA antigens, both from suckling mice and adult hamsters, was diluted with four volumes of 0.85% saline solution and treated by double acetone extraction.[17] The diluted serum was dropped through a 25-gauge needle into 20 volumes of chilled acetone held in an ice-bath while being stirred constantly. Within 10 minutes this mixture was centrifuged at 1,000 rpm for one minute and the supernatant fluid discarded. The sediment was then re-extracted with the same volume of chilled acetone. The mixture was allowed to sit for 30 to 60 minutes before centrifugation at 1,500 rpm for 3 minutes. The resulting sediment was dried under vacuum and resuspended in a volume of borate saline solution (pH 9.0) equal to the diluted serum used for extraction. Before testing, one volume of borate saline solution containing 0.4% bovine albumin was added, and the final antigen, representing a 1:10 dilution of the original serum, was considered as undiluted in determinations of HA activity.

Antigens prepared by the above method were tested for hemagglutinating activity with goose erythrocytes. The mixture had a final pH range of 5.75 to 6.0 at room temperature. Titration of antigens and HI tests were performed in lucite plates, according to the technique described by Clarke and Casals.[12] In HI tests, samples of antiserum were treated with kaolin for removal of nonspecific inhibitors, and serum-antigen mixtures were held overnight at 4°C before addition of goose cells.

RESULTS

Susceptibility of Adult Hamsters to Groups C and Patois Arboviruses

Each virus was inoculated ic or subcutaneously (sc) into a group of two to four 2- to 3-month-old golden hamsters (*Mesocricetus auratus*). As shown in Table 1, the hamsters were susceptible to most group C viruses, and to the Mexican strain of Patois virus, by both routes of inoculation. Illness and death occurred 2 to 4 days after inoculation. In some instances the animals died fewer than 6 hours after the onset of visible illness. Experiments with hamsters of more ad-

TABLE 1

Susceptibility of adult hamsters to groups C and Patois arboviruses

Virus	Passage*	Route of inoculation: Subcutaneous — Illness	Subcutaneous — Incubation interval (*days*)	Intracerebral — Illness	Intracerebral — Incubation interval (*days*)
Apéu	B9	Nil	—	±	4
Caraparú	S7L1B1S1	+	3	+	1–2
Caraparú-like	B4	+	4	+	2
Gumbo-Limbo	B8	+	3	+	3
Itaqui	B3	+	3	+	2
Madrid	B13L6B1	+	3	+	2
Marituba	B11	Nil	—	±	2–3
Murutucú	B3	Nil	—	+	3
Nepuyo (BeAn10709)	L8B1L1	±	2–3	+	3
Nepuyo (63U11)	B4	+	2	+	1–2
Oriboca	B8	+	3	+	3
Ossa	B47	+	3	+	2–3
Patois (BT4971)	B5	Nil	—	Nil	—
Patois (63A49)	B9	+	3	+	3
Restan	B3	+	2	+	2
Zegla	B5	Nil	—	Nil	—

* Number of passages in infant mouse: B, brain; S, serum; L, liver.

vanced age (1 to 2 years) were performed with certain viruses. The pattern of illness and death was similar in all cases, although incubation periods were sometimes prolonged to 5 to 6 days in older animals.

Hemagglutinin Production

As shown in Table 2, viruses that regularly caused death of adult hamsters produced high-titered serum hemagglutinins. Inoculations were first made by the sc route. If HA titers of serum antigens were unsatisfactory, the ic route was tried. The hemagglutinin titers illustrated in Table 2 were obtained in serum harvested at the time of illness. Except for Caraparú virus, all viruses in this group produced high-titered hemagglutinins ranging from 1:640 to 1:5,120. Maximum HA titers of each virus obtained from suckling-mouse serum are shown in the table for comparison. All of these viruses produced HA antigens at the time hamsters became ill, with titers as high as, or higher than, antigens prepared from suckling-mouse serum. In some instances, such as with Madrid and Ossa viruses from Panamá, HA titers from hamsters were 16 times higher than those from mice. Furthermore, the amount of serum obtained from one hamster was about the same as that recovered from 50 to 100 suckling mice. Therefore, an adult hamster furnished as much group C HA antigen as 500 to 1,000 suckling mice.

One- to 2-year-old adult hamsters, which produced more serum, were also tried with Ossa, one of the locally isolated viruses. Antigens were obtained with titers four- to eightfold less than those from animals 8 to 10 weeks of age.

Table 3 shows results for viruses not regularly pathogenic for adult hamsters. Serial bleedings were carried out every 12 hours to determine the

TABLE 2

Serum hemagglutinating antigen of group C and Patois viruses pathogenic to adult hamster

Virus	Route of inoculation	Time of bleeding (*hours*)	Reciprocal HA titer	Reciprocal HA titer from infant-mouse serum
Caraparú	sc	72	80	40
Caraparú-like	ic	43	2,560	320
Gumbo-Limbo	sc	69	1,280	160
Itaqui	ic	48	640	320
Madrid	ic	46	1,280	80
Murutucú	ic	48	1,280	320
Nepuyo (BeAn10709)	ic	43	1,280	80
Nepuyo (63U11)	ic	25	1,280	160
Oriboca	sc	65	5,120	1,280
Ossa	sc	72	1,280	80
Restan	sc	49	1,280	1,280
Patois (63A49)	sc	70	640	320

TABLE 3

Serum hemagglutinating antigen of groups C and Patois viruses nonpathogenic to adult hamster by intracerebral inoculation

Virus	Time of bleeding (*hours*)	Reciprocal HA titer	Reciprocal HA titer from infant-mouse serum
Apéu	60–84	160	40
Marituba	36–48	5,120	1,280
Patois (BT4971)	60–72	640	640
Zegla	24–96	0	320

HA-titer peak. Except for Zegla virus, all agents produced workable HA titers between 36 and 84 hours after inoculation. In attempts to produce hemagglutinins for Zegla virus, sources of virus inocula other than brain, such as infant-mouse liver and mouse and hamster serum, were tried without success.

Relation between Titers of Infectivity and of HA

Patois virus, strain BT 4971, was selected for a detailed study of its pattern of hemagglutinin formation because it did not kill adult hamsters. Animals were bled every 12 hours after injection, and serum obtained for HA antigen was also used for virus titration. Figure 1 demonstrates the consistent direct relation observed between the two parameters, with peak values at 60 hours. This infectivity peak, about $10^{10}LD_{50}$ of virus per milliliter of infected serum, is a truly impressive viremia, more than 100 times the maximum virus concentration detected in infected infant-mouse brain.

Specificity of Hamster Serum HA Antigens

When tested by HI with antigens prepared from suckling-mouse serum, samples of antiserum obtained after two inoculations of virus inhibited four units of homologous hemagglutinin with titers ranging from 1:80 to 1:640. Hamster serum HA antigens were tested with these serum samples at the same time. Results indicated that serum HA antigens prepared from adult hamsters were specifically inhibited by samples of homologous antiserum at titers comparable to those obtained with mouse-serum antigens. Furthermore, relations among pairs of the closely related

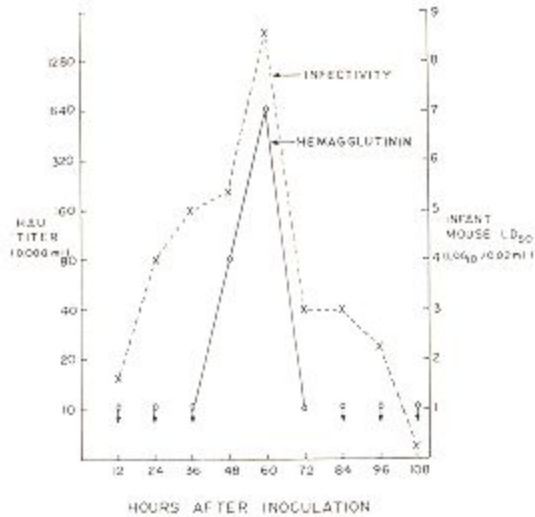

FIGURE 1. Graph showing the infectivity and hemagglutinin responses of adult hamsters infected by the intracerebral route with 10^5TCID_{50} of Patois virus.

viruses obtained with hamster serum antigens were similar to those previously recognized.

Attempts to Prepare Hamster Serum HA Antigens with Arboviruses Other than Groups C and Patois Agents

Results of tests with other arboviruses in adult hamsters are shown in Table 4. Animals were inoculated by the sc or ic route and examined for illness twice daily for 2 weeks. Serum was collected from survivors 3 weeks after inoculation. Among the viruses tested, no HA antigens were found in mouse-brain preparations in our laboratories for Wyeomyia, Cache Valley, Changuinola, Melao, Cocal, and VSV-Indiana. Viruses from which hemagglutinins were not consistently prepared by classic methods included Guamá, Catú, Bimiti, Guaroa, California encephalitis, and Chagres. Others in the study for which good sources of HA antigens were already known included Venezuelan equine encephalomyelitis (VEE), Mucambo, Pixuna, Western equine encephalomyelitis (WEE), Eastern equine encephalomyelitis (EEE), Mayaro, Una, Ilhéus, Bussuquara, and St. Louis encephalitis (SLE).

Bleeding for serum antigens was done at the time of the illness of the hamster or, if nonpathogenic, only once or twice between 24 and 72 hours after inoculation. In contrast to the

TABLE 4

Susceptibility of adult hamsters to new-world arboviruses other than groups C and Patois

Group	Virus and no. of mouse brain passages	Inoculum Log_{10} $TCID_{50}$	No. dead/no. inoc. ic	No. dead/no. inoc. sc	Antibody produced* Test	Antibody produced* ic	Antibody produced* sc
A	VEE P2	7.0	2/2	2/2	—	—	—
	Mucambo P2?	6.5	2/2	2/2	—	—	—
	Pixuna P9	5.5	2/2	0/2	HI†	—	640
	EEE P2	7.5	2/2	2/2	—	—	—
	WEE P5	7.5	2/2	2/2	—	—	—
	Mayaro P4	5.5	0/2	0/2	HI	1,280	320
	Una P11	7.5	0/2	0/2	HI	1,280	320
B	SLE P6	7.0	0/2	0/2	HI	5,120	1,280
	Ilhéus P9	4.5	0/2	0/2	HI	2,650	640
	Bussuquara P12	4.0	0/2	0/2	HI	320	160
Bunyamwera	Cache Valley P12	4.5	2/3	1/3	CF‡	>16	>8
	Guaroa P8	4.0	0/2	0/2	NT§	—	2.5
	Wyeomyia P13	3.5	0/2	0/3	NT	<1.0	<1.0
Guamá	Guamá P5	3.0	1/6	0/2	CF	32	>16
	Catú P6	1.5	0/6	0/2	CF	16	>8
	Bimiti P12	5.5	0/6	0/2	CF	16	16
California encephalitis	California E. P27	2.5	5/5	0/2	NT	—	3.0
	Melao P9	4.5	0/2	0/2	NT	—	3.5
Phlebotomus-fever	Chagres P14	6.0	3/3	4/4	—	—	—
Changuinola	Changuinola P36	3.0	0/2	0/3	CF	16	8
VSV	VSV-Indiana P6	7.5	2/2	1/2	CF	—	>32
	Cocal P3	8.0	3/3	1/3	CF	—	>16

* Reciprocal of serum titer 3 to 4 weeks after inoculation by indicated route.
† HI = hemagglutination inhibition.
‡ CF = complement fixation.
§ NT = neutralization index Log_{10} LD_{50} in infant mice ic.

results obtained with groups C and Patois viruses, all viruses mentioned above produced no HA antigens. Inoculated hamsters surviving the 2-week period were bled several days later and tested for antibodies by standard serologic tests. The data indicated that adult hamsters were infected by all agents employed, with the exception of Wyeomyia virus.

DISCUSSION

From a practical point of view, the preparation of HA antigens for Patois and group C arboviruses from adult-hamster serum has a considerable advantage over that with serum from suckling mice. Large amounts of antigen can be prepared from far fewer animals; therefore, tests for specificity need to be performed less often. Aliquots of this serum antigen can be kept in sealed glass ampules at −70°C over a long period. In many instances it was possible to make additional amounts of antigen from the livers of the same infected hamsters used for serum antigen. Although careful evaluation was not done, limited observation suggested that animals more than 1 year old were satisfactory sources of antigen, with slightly reduced titers partially offset by increased serum yield. If confirmed, this finding indicates an economical use for stock no longer in use for breeding in hamster colonies.

Results of hemagglutinin production, correlated with virus titers in adult-hamster serum, indicated that such animals are highly susceptible to injection with these groups of viruses. Thus, the pattern of infection of groups C and Patois viruses in hamsters was similar to that produced by yellow fever virus in monkeys.[9, 10] Such high-titered hemagglutinins appear to reflect unusually intense viremias associated with hamster infection—a finding worthy of further investigation in connection with studies of the pathogenesis of these agents.

As has been shown in this study, adult hamsters were clinically susceptible to most group C arboviruses, as well as to some group A viruses known to occur in Panamá. These data therefore extend the potential value of the hamster as a

convenient, economical, arbovirus sentinel, a role first demonstrated by Scherer *et al.* for VEE virus[39] and confirmed by Srihongse *et al.*[41] Although studies should be repeated with small doses of low- or unpassaged virus, it is evident that many other arboviruses induce a silent infection in the hamster. Thus, this animal may prove to be a valuable sentinel for detection of arbovirus activity, yielding virus strains of pathogenic groups C, A, and Patois viruses, and serologic evidence of infection by other agents whose arthropod vectors are attracted to feed on it.

SUMMARY

Most group C arboviruses produced signs of illness and death in 2- to 3-month-old hamsters within a few days of infection. Serum obtained from these animals 2 to 4 days after inoculation possessed hemagglutinins in titers of 1:1,280 to 1:5,120 for Caraparú-like, Gumbo Limbo, Madrid, Marituba, Murutucú, Nepuyo, Oriboca, Ossa, Restan, and the Mexican strain of Nepuyo (63U11) viruses. Hemagglutinin titers of 1:80 to 1:640 were obtained with Apéu, Caraparú, and Itaqui viruses. Among Patois group agents, both Panamanian and Mexican strains of Patois virus produced HA with titers of 1:640, but Zegla virus did not induce hemagglutinins. Antigens prepared from hamster serum demonstrated specificity in HI tests comparable to those from suckling-mouse serum. Hamsters inoculated with arboviruses other than groups C and Patois, such as VEE, Mucambo, EEE, Pixuna, WEE, Una, Mayaro, SLE, Ilhéus, Bussuquara, Cache Valley, Guaroa, Wyeomyia, Guamá, Catú, Bimiti, Chagres, Changuinola, VSV-Indiana, Cocal, California encephalitis, and Melao, did not produce serum HA antigens. Some of these viruses produced illness and death in hamsters. Others did not, although antibodies developed during convalescence.

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REFERENCES

1. Causey, O. R., Causey, C. E., Maroja, O. M., and Macedo, D. G., 1961. The isolation of arthropod-borne viruses, including members of two hitherto undescribed serological groups, in the Amazon region of Brazil. *Am. J. Trop. Med. & Hyg., 10:* 227–249.
2. Casals, Jordi, and Whitman, Loring, 1961. Group C, a new serological group of hitherto undescribed arthropod-borne viruses. Immunological studies. *Am. J. Trop. Med. & Hyg., 10:* 250–258.
3. Shope, R. E., Causey, C. E., and Causey, O. R., 1961. Itaqui virus, a new member of arthropod-borne group C. *Am. J. Trop. Med. & Hyg., 10:* 264–265.
4. Jonkers, A. H., Spence, L., Downs, W. G., and Worth, C. B., 1964. Laboratory studies with wild rodents and viruses native to Trinidad, II. Studies with the Trinidad Caraparu-like agent TRVL 34053-1. *Am. J. Trop. Med. & Hyg., 13:* 728–733.
5. Spence, L., Anderson, C. R., Aitken, T. H. G., and Downs, W. G., 1966. Nepuyo virus, a new group C agent isolated in Trinidad and Brazil. I. Isolation and properties of the Trinidadian strain. *Am. J. Trop. Med. & Hyg., 15:* 71–74.
6. Jonkers, A. H., Metselaar, D., Pães de Andrade, A. H., and Tikasingh, E. S., 1967. Restan virus, a new group C arbovirus from Trinidad and Surinam. *Am. J. Trop. Med. & Hyg., 16:* 74–78.
7. Rodaniche, E. de, Pães de Andrade, Amelia, and Galindo, Pedro, 1964. Isolation of two antigenically distinct arthropod-borne viruses of group C in Panama. *Am. J. Trop. Med. & Hyg., 13:* 839–843.
8. Scherer, W. F. Cornell University Medical College, 1300 York Avenue, New York, N. Y. 10021. Personal communication, 1967.
9. Fields, B. N., Henderson, B. E., Coleman, P. H., Calisher, C. H., and Work, T. H., 1967. Isolation and characterization of three viruses serologically related to group C and/or Guamá arboviruses. *Bact. Proc., 67:* 166–167.
10. Srihongse, Sunthorn, Galindo, Pedro, and Grayson, M. A., 1966. Isolation of group C arboviruses in Panamá including two new members, Patois and Zegla. *Am. J. Trop. Med. & Hyg., 15:* 379–384.
11. Srihongse, Sunthorn, and Shope, R. E., 1968. The Patois group of arboviruses. *Acta Virol., 12:* 453–456.
12. Arboviruses and human disease, 1967. *World Health Organ. Tech. Rep. Ser., 369,* 84 pp.
13. Shope, R. E., and Causey, O. R., 1962. Further studies on the serological relationships of group C arthropod-borne viruses and the application of these relationships to rapid identification of types. *Am. J. Trop. Med. & Hyg., 11:* 283–290.

14. Buckley, S. M., Pinheiro, F. P., and Clarke, D. H., 1963. Hemagglutinin formation by group C viruses in HeLa (Gey) cells. *Anais de Microbiol. 11:* 183–186.

15. Zarate, M. L., Geiger, R. H., Shope, R. E., and Scherer, W. F., 1968. Intergroup antigenic relationships among arboviruses manifested by a Mexican strain of Patois virus and viruses of the Bunyamwera, C, California, Capim and Guama groups. *Am. J. Epidemiol., 88:* 273–286.

16. Reed, L. J., and Muench, H., 1938. A simple method of estimating fifty per cent endpoints. *Am. J. Hyg., 27:* 493–497.

17. Clarke, D. H., and Casals, J., 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am. J. Trop. Med. & Hyg., 7:* 561–573.

18. Stokes, Adrian, Bauer, J. H., and Hudson, N. P., 1928. Experimental transmission of yellow fever to laboratory animals. *Am. J. Trop. Med., 8:* 103–164.

19. Bauer, J. H., 1931. Some characteristics of yellow fever virus. *Am. J. Trop. Med., 11:* 337–353.

20. Scherer, W. F., Dickerman, R. W., Wong Chia, C., Ventura, A., Moorehouse, A., Geiger, R., and Najera, A. D., 1964. Venezuelan equine encephalitis virus in Veracruz, Mexico, and the use of hamsters as sentinels. *Science, 145:* 274–275.

21. Srihongse, Sunthorn, Scherer, W. F., and Galindo, Pedro, 1967. Detection of arboviruses by sentinel hamsters during the low period of transmission. *Am. J. Trop. Med. & Hyg., 16:* 519–524.