APPLIED MICROBIOLOGY, May 1972, p. 908-913

Vol. 23, No. 5
Printed in U.S.A.

Yellow Fever Vaccine

V. Antibody Response in Monkeys Inoculated with Graded Doses of the 17D Vaccine

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Received for publication 10 January 1972

A dosage equal to or greater than approximately 3.4 Dex (decimal exponent, $\log_{10}$) weanling mouse intracerebral 50% lethal dose (LD_{50}) was sufficient to elicit a yellow fever antibody response, as determined by the plaque neutralization (PN) test, in better than 90% of vaccinated rhesus monkeys. Lower dosages were progressively less effective in terms of PN titers and the PN and hemagglutination-inhibition serological conversion rates observed. A dose of between 3.4 and 4.2 Dex weanling mouse intracerebral LD_{50}, or one-tenth to one times the dosage recommended for man, provided an optimal antibody response in monkeys. In rhesus monkeys, in contrast to the findings for man, pre-existing yellow fever antibody did not interfere with the antibody response to yellow fever vaccine. The PN test was felt to be a more sensitive and specific indicator of yellow fever antibody in rhesus monkeys after vaccination than the hemagglutination inhibition or complement fixation tests.

The recent development of avian leukosis virus (ALV)-free yellow fever (YF) vaccine seed viruses (2, 14) and the importance of active immunization in the control of epidemic YF prompted a re-evaluation of the parameters of vaccine-induced YF immunity. At the onset of our studies, it became obvious that there was a need to update the methodology since most of the earlier studies on vaccine-induced immunity were performed during the late 1930's and early 1940's, a time when tissue culture techniques were in their infancy and some serological procedures [e.g., cell culture neutralization and hemagglutination inhibition (HI) tests] were not yet developed. During the course of our studies, a sensitive and reproducible YF virus plaque neutralization (PN) test was developed (9) and employed from the outset in the studies mentioned above (9, 14, 15) and in our subsequent studies on the antigenicity of the ALV-free YF vaccine in rhesus monkeys (16) and in man (11). In these latter studies a nearly constant high dose of vaccine was employed. It seemed prudent to determine the dose limits of this vaccine-induced immunity. This report deals with our evaluation of the effect of graded doses of the ALV-free 17D YF vaccine upon the antibody response in rhesus monkeys.

MATERIALS AND METHODS

Vaccines. ALV-free YF vaccine lot number 6678, manufactured by the National Drug Co., was used throughout this study. The numbers of monkeys inoculated and the dosage administered to each group of monkeys are shown in Table 1. Freeze-dried vaccine was reconstituted with sterile 0.85% saline solution and used within a 2-hr period. Immediately prior to inoculation, the appropriate serial 10-fold dilution in the same saline diluent was prepared. To determine the actual virus dose inoculated into monkeys, each virus dilution was titrated in both newborn and weanling mice. The diluent used in these titrations was Dulbecco's phosphate-buffered saline solution (3) containing 0.5% bovine plasma albumin.

Viruses. The method of preparation of the infectious stock YF virus (17D strain) used in the PN test has been described (9).

Antigens. The hemagglutination (HA) antigens were prepared by sucrose-acetone extraction (1) of suckling mouse brains harvested when animals sickened.

Animals. Indian rhesus monkeys (*Macaca mulatta*) weighing 3 to 7 lb. (approximately 1.4 to 3.2 kg) were inoculated intramuscularly with 0.5 ml of YF vaccine on the day of their arrival (groups 1 and 2) or within 10 days after their arrival (group 3) at the Primate Quarantine Unit, Laboratory Aids Branch, National Institutes of Health (NIH).

Newborn mice (NBM; 1 to 3 days old) and wean-

ling mice (WM; 16 to 20 g, 24 to 28 days old; Swiss albino, NIH General Purpose strain) used for titrating the vaccine inoculum were obtained from the Rodent and Rabbit Production Section, Laboratory Aids Branch, NIH. In our studies, complete litters (containing 6 to 16 NBM) and 10 WM were used at each dilution point; 0.03 ml was inoculated by the intracerebral route, and 50% lethal dose (LD_{50}) end points were determined at 21 days by the Kärber method (6) and expressed as Dex values (4).

Cell cultures. The MA-104 embryonic rhesus monkey kidney cell cultures (originally obtained from Microbiological Associates, Inc., Bethesda, Md.) were prepared in a routine manner established by the cell biology section of our laboratory. The method of preparation has been described (9).

Serological studies. Sera were obtained from all monkeys prior to (pre) and at 26 to 31 days (4 wk) and 55 to 60 days (8 wk) after inoculation with YF vaccine. Sera were kept at 4 C from the time of blood collection and during processing until the time of final storage at −20 C. All complete sets of sera (pre, 4 wk, and 8 wk) from each monkey were tested by means of the PN, HI, and complement fixation (CF) procedures described below. Tests were set up so that all serum samples from a given monkey were tested simultaneously. Each serological test included samples of serum from recipients of each vaccine dilution.

PN test. The test utilizing the constant serum-varying virus dilutions technique has previously been described (10). The difference in titer between pre- and postimmunization serum samples represents the neutralizing capacity of the serum and is expressed as the neutralization index (NI) in this paper.

HI test. The basic procedure of Clarke and Casals (1) modified for use in Microtiter equipment (7) was used as described previously (13). The highest dilution of serum which completely inhibited HA was recorded as the serum antibody titer.

CF test. The CF procedure used has been described previously (13). Five 50% hemolytic units of complement and four to eight units of antigen were added to serial twofold dilutions of heat-inactivated (56 C for 30 min) sera; the hemolytic system was added after incubation at 4 C for 18 hr, and the mixture was incubated at 37 C for 30 min with shaking of the plates at 10 and 20 min.

RESULTS

Immunization of monkeys. Table 1 shows the numbers of monkeys and potency of the YF vaccine dilutions inoculated into the animals. In each of three separate experiments, undiluted and serial 10-fold dilutions (10^{-1} to 10^{-5}) of vaccine were inoculated into groups of approximately 14 monkeys. The data in Table 1 represent a summary of the three experiments. From the titrations of the undiluted and the 10^{-1} to 10^{-3} dilutions of inocula, it would appear that there should be virus in the 10^{-4} and 10^{-5} dilutions.

TABLE 1. *Numbers of monkeys and vaccine dosages used*

Group no.	Vaccine[a] dilution	No. of monkeys inoculated[b]	Vaccine potency	
			NBM[c]	WM[d]
1	Undiluted	48	5.9[e]	5.2
2	10^{-1}	42	5.2	4.2
3	10^{-2}	42	4.4	3.4
4	10^{-3}	42	2.7	2.2
5	10^{-4}	42	0	0
6	10^{-5}	42	0	0

[a] Yellow fever vaccine lot no. 6678.
[b] Total = 258.
[c] NBM = newborn (1 to 3 days old) mice.
[d] WM = weanling (16 to 20 g; 24 to 28 days old) mice.
[e] Mouse intracerebral LD_{50}, expressed as Dex values (4), per 0.5 ml of inoculum used to vaccinate monkeys; each value represents the average value of three tests.

Antigenicity of YF vaccine in monkeys. The results of the PN tests are shown in Table 2. None of the monkeys used had pre-existing PN antibody to YF virus. Essentially comparable group mean antibody titers and antibody conversion rates were seen in groups 1 through 3 (10^{0}, 10^{-1}, and 10^{-2} dilutions, respectively) receiving the largest vaccine dosages. Antibody conversion rates in these groups ranged from 94 to 100% and indicated the high degree of immunogenicity of dosages containing virus titers equal to or greater than 3.5 Dex. Progressively lower PN titers and antibody conversion rates were seen in groups 4, 5, and 6 (10^{-3}, 10^{-4}, and 10^{-5} dilutions, respectively). The PN and per cent conversion values for each of these groups were significantly lower (5% level) than the corresponding values for the group receiving the next higher vaccine dosage level. There appeared to be no real differences between PN titers and conversion rates at 4 and 8 weeks. The antibody conversion of groups 5 and 6 indicates that there was virus in the 10^{-4} and 10^{-5} inocula. From the per cent antibody responses shown in Table 2, the titer of 17D virus in the vaccine lot employed in monkey intramuscular 50% infective doses (ID_{50}) is calculated to be 3.9 Dex per 0.5 ml of inoculum.

The results of the HI and CF tests are shown in Table 3. The HI test was less sensitive than the PN test for detecting positive reactors following vaccination. Approximately 50% of antibody conversions detected by the PN test would have gone undetected in the HI test. Positive reactions to YF antigen in monkey

TABLE 2. *Effect of graded doses of the yellow fever 17D vaccine upon the plaque-neutralizing (PN) antibody titers of vaccinated monkeys*

Vaccine group	Vaccine dilution	No. of monkeys tested[a]	PN antibody response at:			
			4 wk		8 wk	
			NI[b]	%	NI	%
1	Undiluted	39	2.7[c] ± 0.2	97.4[d]	2.7 ± 0.2	97.3[d]
2	10^{-1}	37	2.9 ± 0.2	100	2.5 ± 0.2	97.2
3	10^{-2}	33	2.6 ± 0.2	97.0	2.7 ± 0.2	93.8
4	10^{-3}	35	2.0 ± 0.2*[e]	80.0*	1.9 ± 0.2*	80.0*
5	10^{-4}	38	1.2 ± 0.2*	50.0*	1.3 ± 0.2*	50.0*
6	10^{-5}	39	0.46 ± 0.1*	15.8*	0.58 ± 0.2*	18.4*

[a] Each value represents total number of monkeys employed in three immunization experiments. A total of 221 monkeys were used for all experiments.
[b] Neutralization index ± standard error.
[c] Average values of three trials, expressed as Dex values (4).
[d] Per cent of monkeys developing an LNI $\geq$ 0.7.
[e] Asterisk indicates a significant difference, at the 5% level, from the value immediately above it in the table.

TABLE 3. *Effect of graded doses of the yellow fever 17D vaccine upon the hemagglutination inhibition (HI) and complement fixation (CF) antibody response of vaccinated monkeys*

Vaccine group	Vaccine dilution	HI			CF		
		No. of monkeys tested	Per cent positive[a]		No. of monkeys tested	Per cent positive[a]	
			4 wk	8 wk		4 wk	8 wk
1	Undiluted	20 (15)[b]	55 (53)[c]	25 (33)[c]	18 (15)[d]	83 (100)[e]	78 (93)[e]
2	10^{-1}	21 (14)	52 (43)	48 (43)	7 (7)	100 (100)	86 (86)
3	10^{-2}	15 (14)	33 (36)	53 (57)	18 (14)	81 (100)	67 (86)
4	10^{-3}	14 (11)	57 (54)	36 (36)	19 (13)	84 (92)	58 (77)
5	10^{-4}	17 (16)	29 (25)	29 (31)	10 (6)	50 (83)	50 (83)
6	10^{-5}	14 (13)	7 (8)	14 (15)	10 (9)	60 (56)	60 (67)

[a] Per cent developing a fourfold or greater rise in antibody titer.
[b] Number having no HI antibody (i.e., $\lesssim$1:2) to 17D strain YF virus in preinoculation serum. Total number tested = 101.
[c] Per cent not having YF HI antibody in preinoculation serum who developed a fourfold or greater rise in HI antibody titer.
[d] Number having no CF antibody (i.e., $\lesssim$1:2) to 17D strain YF virus in preinoculation serum. Total number tested = 82.
[e] Per cent not having YF CF antibody in preinoculation serum who developed a fourfold or greater rise in CF titer.

sera prior to vaccination were believed due primarily to cross-reacting West Nile (WN) or Dengue antibody and did not alter the antibody response to YF vaccination. HI responses at 4 and 8 weeks postinoculation were similar.

Antibody conversion after vaccination appeared to be readily detected by means of the CF test (Table 3). The CF test, nevertheless, was less sensitive than the PN test. The high per cent of reactors recorded in monkeys which had received the 10^{-5} dilution of vaccine (group 6) is difficult to explain. Indeed, three of the six monkeys in group 6, whose titers increased fourfold after vaccination, had pre-existing HI titers against WN antigen. Nearly two-thirds of all the CF-negative monkeys responding to the 17D vaccine with fourfold or greater titer increases showed pre-existing antibody to one or more of the arbovirus antigens employed in the HI test. In contrast, only 8% of HI-negative monkeys responding positively to YF vaccine were found to have pre-existing CF antibody.

Arbovirus HI antibodies in prevaccination sera. As previously stated, the monkeys used in this study had a moderate incidence of pre-existing antibody to certain group B arboviruses, as shown in Table 4. All pre-inoculation

sera reacting to the 17D antigen also reacted to WN antigen, suggesting that the YF HI antibody in these sera most likely represented cross-reactions to group B viruses indigenous to India, such as WN and Dengue. Eighteen monkeys had pre-existing antibody to Semliki forest (SF) virus; 11 of these were seen in sera that also contained pre-existing antibody to one or more of the group B arboviruses without antibody to Chikungunya virus. Two of the SF-positives were positive for both group B and Chikungunya viruses, and five monkeys had pre-existing SF HI antibody only. Six pre-sera were positive for Bunyamwera antibody; such positives were always seen in association with pre-existing YF and WN antibody. Three pre-sera, two in association with SF antibody, were positive for Chikungunya antibody. Langat HI antibody was not detected in pre-sera from these monkeys.

HI antibody response to several arbovirus antigens after inoculation with YF vaccine. After administration of YF vaccine, serological tests were performed with several arbovirus antigens to determine (i) the effect of pre-existing antibody upon the antibody response to YF vaccine and (ii) the effect of YF vaccination upon eliciting an antibody response broadly reacting to other arboviruses. The results shown in Table 5 demonstrate that pre-existing WN antibody had no effect upon the response to YF vaccine. Too few monkeys had pre-existing antibody to the other arboviruses to determine the effect of this parameter upon the response to YF vaccine.

Administration of YF vaccine did elicit antibody capable of reacting with WN, but not with Langat, HA antigens.

The effect of pre-existing WN HI antibody upon the development of 17D HI antibody following vaccination is further detailed in Table 6. The results demonstrate that pre-existing WN antibody had no effect on the HI antibody response to YF vaccine; chi-square analysis indicated no significant differences between numbers of positive reactors (at the 5% level of significance) within any of the dilution groups or between the total numbers of reactors in the positive and negative WN pre-serum groups.

DISCUSSION

The results of these studies indicate that a dosage equal to or greater than approximately 3.4 Dex WM intracerebral LD_{50} is able to elicit a YF antibody response, as determined by the PN test, in better than 90% of inoculated rhesus monkeys. Lower dosages were progressively less effective in terms of the PN titers and the PN and HI serological conversion rates that could be anticipated after vaccination. A dose of between 3.4 and 4.2 Dex WM intracerebral LD_{50}, or from one-tenth to one times the dosage recommended for man, would therefore be selected to provide an optimal antibody response in rhesus monkeys.

The present PN test data confirm the previous findings (16) indicating the high degree of immunogenicity of the ALV-free 17D vaccine in monkeys.

Our findings also verify and extend the earlier observation (16) indicating that in monkeys pre-existing cross-reacting antibodies to antigenically related arboviruses do not interfere with the antibody response to YF vaccine. These observations in rhesus monkeys are in contrast to what was observed in man, where

TABLE 4. *Number and per cent of monkeys with hemagglutination inhibition antibodies to several arboviruses in prevaccination sera*

Vaccine group	Vaccine dilution	No. of monkeys tested	Arbovirus antigens[a]						
			YF (17D)	YF (FN)	WN	Langat	SF	Chik	Bunyam
1	Undiluted	20	5[b]	9	12	0	6	1	1
2	10^{-1}	21	7	7	12	0	6	1	1
3	10^{-2}	15	1	2	6	0	0	1	2
4	10^{-3}	14	3	5	5	0	3	0	0
5	10^{-4}	17	1	1	1	0	0	0	1
6	10^{-5}	14	1	3	9	0	0	0	1
Total		101	18	27	45	0	18	3	6
Per cent		100	18	27	45	0	18	3.0	6.0

[a] Abbreviations: YF, yellow fever; WN, West Nile; SF, Semliki forest; Chik, Chikungunya; Bunyam, Bunyamwera.

[b] Number of monkeys demonstrating hemagglutination inhibition antibody to respective antigen in their pre-vaccination sera.

TABLE 5. *Per cent of monkeys developing a fourfold or greater rise in hemagglutination inhibition (HI) antibody after inoculation with yellow fever vaccine*

Vaccine group	Vaccine dilution	Antibody status of preserum	Arbovirus antigens[a]													
			YF (17D)		YF (FN)		WN		Langat		SF		Bunyam		Chik	
			4 wk	8 wk	4 wk	8 wk	4 wk	8 wk	4 wk	8 wk	4 wk	8 wk	4 wk	8 wk	4 wk	8 wk
1	Undiluted	Pos.	60[b]	0	44	33	8.3	0	—	—	0	0	0	0	0	0
		Neg.	53[c]	33	45	18	12	25	0	0	0	0	0	0	0	0
2	10^{-1}	Pos.	71	57	71	57	8.3	0	—	—	0	0	0	0	0	0
		Neg.	43	43	36	36	11	0	0	0	0	0	0	0	0	0
3	10^{-2}	Pos.	0	0	0	0	0	0	—	—	—	—	0	0	0	0
		Neg.	36	57	31	62	0	11	0	0	0	0	0	0	0	0
4	10^{-3}	Pos.	67	33	20	20	20	0	—	—	0	0	—	—	—	—
		Neg.	54	36	55	55	0	0	0	0	0	0	0	0	0	0
5	10^{-4}	Pos.	100	0	100	0	0	0	—	—	—	—	0	0	—	—
		Neg.	25	31	19	25	0	0	0	0	0	0	0	0	0	0
6	10^{-5}	Pos.	0	0	0	0	0	0	—	—	0	0	0	0	—	—
		Neg.	7.7	15	9.1	9.1	0	0	0	0	0	0	0	0	0	0
Total		Pos.	61	28	41	30	6.7	0	—	—	0	0	0	0	0	0
		Neg.	36	36	31	34	3.6	5.4	0	0	0	0	0	0	0	0

[a] Abbreviations: YF, yellow fever; WN, West Nile; SF, Semliki forest; Bunyam, Bunyamwera; Chik, Chikungunya.

[b] Per cent of monkeys with pre-existing antibody to an antigen developing a fourfold or greater rise in HI antibody to the same antigen; these percentages multiplied by the number of monkeys either positive or negative for pre-existing antibody in the corresponding vaccine and arbovirus group (shown in Table 4) will give the number of animals developing a fourfold or greater response.

[c] Per cent of monkeys with no pre-existing antibody to an antigen developing a fourfold or greater rise in HI antibody titer to the same antigen.

TABLE 6. *Effect of pre-existing West Nile (WN) hemagglutination inhibition (HI) antibody on the development of antibody to 17D yellow fever virus vaccine*

Vaccine group	Vaccine dilution	Postvaccination response					
		Pre-serum WN positive[a]			Pre-serum WN negative[b]		
		No. WN pos.	No. 17D pos.[c]	Per cent 17D pos.	No. WN neg.	No. 17D pos.	Per cent 17D pos.
1	Undiluted	12	10	83	8	5	62
2	10^{-1}	12	9	75	9	4	44
3	10^{-2}	6	4	67	9	5	56
4	10^{-3}	5	3	60	9	7	78
5	10^{-4}	1	1	100	16	5	31
6	10^{-5}	9	2	22	5	0	0
Total		45	29		56	26	
Per cent		45	64		55	46	

[a] HI at a serum dilution of 1:4 or greater.

[b] No HI above a serum dilution of 1:2.

[c] Number developing a fourfold or greater rise in HI antibody.

pre-existing YF antibody appeared to interfere with the antibody response to YF vaccine (11). This apparent discrepancy might be explained by the data which suggest that YF antibody in rhesus monkeys was not specifically induced by previous exposure to YF virus, but rather represented cross-reactions from antibody induced by previous exposure to other group B arboviruses indigenous to that part of India where the rhesus monkeys habitated. On the contrary, the pre-existing YF antibody observed in volunteers in an earlier study (11) was probably due to previous YF vaccination, and this specific YF antibody would more likely be expected to interfere with repeat vaccinations.

The presence of pre-existing YF HI antibody only in monkeys with WN antibody supports the belief (16) that such pre-serum YF titers represent cross-reactions to other group B ar-

boviruses. West Nile HI antibody occurred in pre-serum with a markedly greater frequency than did YF-reactive antibody.

It was surprising to observe the high CF antibody conversion rates in YF-vaccinated monkeys since development of CF antibody does not ordinarily follow peripheral inoculation of the 17D vaccine in man (8). CF antibody does occur regularly in individuals following attacks of disease caused by wild-type virus (5, 8) and in persons developing signs and symptoms of encephalitis following receipt of 17D vaccine (5, 8). The CF response observed in monkeys following peripheral inoculation of 17D vaccine may be explained on the basis of species variation. It is possible that previous experience with other related group B arboviruses may affect the CF antibody response to YF vaccine. We do not think that this would explain the differences observed between the responses of monkeys and man, because the high CF antibody conversion rates observed in the former were unrelated to their previous experience with group B arbovirus diseases related to the three antigens (i.e., YF, WN, and Langat) employed in the HI tests, and because volunteers having pre-existing group B arbovirus HI antibody did not develop a CF antibody response to 17D vaccine (11).

Additional studies, using updated methodology, on the effect of graded vaccine dosages upon the antibody response in man would appear worthwhile to determine the dosage limits of the 17D vaccine-induced response.

ACKNOWLEDGMENTS

The authors thank the Primate Quarantine Unit, Laboratory Aids Branch, NIH, for their assistance in conducting the monkey inoculations, the Cell Biology Section of our laboratory for providing the cell cultures, Martin G. Myers and Alan Ve Richman for their assistance, and Walter C. Browne and Carolyn Jackson for their technical help in these experiments.

LITERATURE CITED

1. Clarke, D. H., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. Hyg. **7**: 561-573.
2. Draper, C. C. 1967. A yellow fever vaccine free from avian leukosis viruses. J. Hyg. **65**:505-513.
3. Dulbecco, R., and M. Vogt. 1954. Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med. **99**:167-199.
4. Haldane, J. B. S. 1960. Dex or order of magnitude? Nature (London) **187**:879.
5. Fox, J. P., E. H. Lennette, C. Manso, and J. R. Souza Aguiar. 1942. Encephalitis in man following vaccination with 17D yellow fever virus. Amer. J. Hyg. **36**:117-142.
6. Lennette, E. H. 1969. General principles underlying laboratory diagnosis of viral and rickettsial infections, p. 1. *In* Diagnostic procedures for viral and rickettsial diseases, 4th ed. Amer. Pub. Health Ass., Inc., New York.
7. Sever, J. L. 1962. Application of a microtechnique to viral serological investigations. J. Immunol. **88**:320-329.
8. Smithburn, K. C. 1951. Immunology, chapter 4, p. 165. *In* G. K. Strode (ed.), Yellow fever. McGraw-Hill Book Co., Inc., New York.
9. Spector, S. L., and N. M. Tauraso. 1968. Yellow fever virus. I. Development and evaluation of a plaque neutralization test. Appl. Microbiol. **16**:1770-1775.
10. Spector, S. L., and N. M. Tauraso. 1969. Yellow fever virus. II. Factors affecting the plaque neutralization test. Appl. Microbiol. **18**:736-743.
11. Tauraso, N. M., R. L. Coultrip, L. J. Legters, A. V. Richman, D. M. Rosenberg, T. O. Savadge, A. Shelokov, S. L. Spector, and R. W. Trimmer. 1972. Yellow fever vaccine. IV. Reactogenicity and antibody response in volunteers inoculated with a vaccine free from contaminating avian leukosis viruses. Proc. Soc. Exp. Biol. Med. **139**:439-446.
12. Tauraso, N. M., M. G. Myers, E. V. Nau, T. C. O'Brien, S. S. Spindel, and R. W. Trimmer. 1972. Effect of interval of inoculation upon antigenicity of live smallpox and yellow fever vaccines in man. J. Infect. Dis., *in press*.
13. Tauraso, N., and A. Shelokov. 1965. Protection against Junin virus by immunization with live Tacaribe virus. Proc. Soc. Exp. Biol. Med. **119**:608-611.
14. Tauraso, N. M., S. L. Spector, W. G. Jahnes, and A. Shelokov. 1968. Yellow fever vaccine. I. Development of a vaccine seed free from contaminating avian leukosis viruses. Proc. Soc. Exp. Biol. Med. **127**:1116-1120.
15. Tauraso, N. M., S. L. Spector, R. L. Kirschstein, E. B. Seligmann, Jr., and J. F. Farber. 1969. Yellow fever vaccine. II. Antigenicity and neurovirulence of a vaccine seed free from avian leukosis virus. Appl. Microbiol. **17**:866-870.
16. Tauraso, N. M., S. L. Spector, and R. W. Trimmer. 1972. Yellow fever vaccine. III. Antibody response in monkeys inoculated with a vaccine free from contaminating avian leukosis viruses. Arch. Gesamte Virusforsch. Vol. 37.