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Neutralizing and Haemagglutination-Inhibiting Antibodies to Yellow Fever 17 Years after Vaccination with 17D Vaccine*

HERNANDO GROOT[1] & RUBEM BAHIA RIBEIRO[2]

The duration of immunity conferred by yellow fever vaccine is as yet undetermined. In this study the neutralizing and haemagglutination-inhibiting antibodies to yellow fever were investigated in 108 persons living in Pouso Alegre, Brazil, where yellow fever has never been reported. These persons had been vaccinated with 17D yellow fever vaccine between 27 December 1940 and 5 February 1941, but not again; and their antibody pattern was compared with that of 78 controls who had never been vaccinated. In the vaccinated group the majority had neutralizing and haemagglutination-inhibiting antibodies present in the serum, whereas in the unvaccinated group antibodies were all but completely absent. Heterologous antibodies to other viruses of Casal's Group B were also found, and the significance of this finding is discussed.

In the uplands of the south-eastern part of the State of Minas Gerais, Brazil, there is an appreciable area in which yellow fever has never been recognized, and in which several studies of 17D yellow fever vaccine have been made. The studies have comprised alternative methods of applying the vaccine, and studies of the duration of immunity following vaccination, at two and four years (Fox & Cabral, 1943), at six years (Fox, Da Cunha & Kossobudzki, 1948), and now at 17 years, after vaccination.

The *Aëdes aegypti* mosquito has never been found in the area around the town and municipio (county, or commune) of Pouso Alegre, in Minas Gerais, presumably because of the relatively mild atmospheric temperatures due to the altitude of about 1100 m. Presumably also because of the altitude, jungle yellow fever has never been recognized in the area.

The purpose of this paper is to present the results of studies of the amount of yellow fever neutralizing antibody in persons resident in Pouso Alegre 17 years after they were vaccinated with 17D vaccine. The vaccination was carried out between 27 December 1940 and 5 February 1941 (Fox, Kossobudzki & Da Cunha, 1943). Fifteen sister lots of vaccine were used, all of them known to have had a high titre of virus shortly before use. In addition, tests on sera taken from 918 persons one month after vaccination showed that all had developed demonstrable yellow fever neutralizing antibody. The experimental vaccinations were performed in a manner probably significantly better than that of the average routine vaccinations.

The blood specimens were collected in September 1958 (by R. B. Ribeiro). The first examination of the sera was done in the laboratory of Dr Hugo W. Laemmert of the Oswaldo Cruz Institute, Río de Janeiro. Some of the sera were then re-examined by sensitive techniques for the presence of neutralizing antibody at the Carlos Finlay Institute, Bogotá, Colombia (by H. Groot). Staff members of the Pan American Health Organization provided liaison between the workers in South America.

MATERIAL AND METHODS

Collection of blood specimens

In the period from 23 to 30 September 1958, 108 specimens of blood were collected from individuals whose names were recorded in vaccination registration books available from the 1940-41 studies, during which a total of 5275 persons had been vaccinated.

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[1] Director, Laboratory of the Carlos Finlay Institute, Bogotá, Colombia.

[2] National Department of Rural Endemic Diseases, Ministry of Health, Brazil.

The age and sex of the donors of blood in 1958 were carefully checked against the entries in the books. In addition, bloods were taken from 78 persons between 7 and 16 years of age, born after the 1941 vaccinations had been completed. It is particularly noteworthy that no yellow fever vaccinations had been performed in the Pouso Alegre area between 1941 and the time when the 1958 specimens were taken. Nobody in this second group was bled unless a responsible adult gave assurance that the child had not been vaccinated. This group of bloods was taken in order to provide a control on the absence of yellow fever from the area after 1941.

From each of the persons bled about 15 ml of blood were taken, using a separate 30-ml Bayer Venule (a vacuum syringe with an attached needle) for each person. The following morning, after the blood specimens had clotted and settled, the sera were removed with separate 15-ml Venules under aseptic conditions. The pertinent data regarding each person bled were recorded, and a "local number" was given to each blood specimen as it was taken. As each serum specimen was transferred from the large Venule to the small one, the adhesive plaster label bearing the local number was also transferred. The 186 serum specimens were sent in a single shipment to the Oswaldo Cruz Institute in the 15-ml Venules.

Neutralization tests for yellow fever done at the Oswaldo Cruz Institute

All the sera were tested for the presence of yellow fever neutralizing antibody at the Oswaldo Cruz Institute (OCI) by the intracerebral technique (NT) in weanling mice inoculated with 0.03 ml of serum virus mixtures, using six mice per serum. The sera were tested in three different "runs", each of which contained both post-vaccination sera and sera from presumably unvaccinated children. The LD_{50} of French neurotropic (FN) virus used in the three runs was 40.5, 107, and 125, as determined by virus titrations in each run. The technique used in these runs is considered to be the standard technique for yellow fever neutralization tests, making them highly specific for yellow fever neutralizing antibody, or "severe". Survival ratios (SR's) of 4/6, 5/6 or 6/6 obtained in a single test were interpreted to mean that the serum contained a demonstrable amount of yellow fever neutralizing antibody. The numerator of the SR fractions is the number of mice that survived ten days, while the denominator is the number of mice alive on the fourth day of the test.

Neutralization tests for yellow fever done at the Carlos Finlay Institute

Part of the sera that were first tested at the OCI were sent to the Carlos Finlay Institute (CFI) to be examined by the "sensitive" neutralization test technique that is frequently used there in the study of sera from persons vaccinated with 17D yellow fever virus. The neutralization test is made sensitive by the use of the accessory serum factor that is present in fresh undiluted serum of a number of different animal species, including man, rhesus monkeys and guinea-pigs.

The test was done by the intracerebral technique in Swiss mice 20-40 days of age, 8 mice being inoculated with each serum-virus mixture. The FN virus was diluted in bovine albumin-saline except for the final tenfold dilution, which was made in undiluted fresh normal guinea-pig serum. The mixtures of equal parts of test serum and virus dilution were incubated at 37°C for 60 minutes. All the sera were tested in a single run against FN virus, the LD_{50} of which, per mouse, was 69.

The sera tested comprised 39 post-vaccination sera, on which SR's of 0/6, 1/6, 2/6 or 3/6 had been obtained in the standard tests done at the OCI, plus 7 sera which had SR's of 6/6, these latter serving as a control on the positive results. In addition, 36 sera from presumably unvaccinated children were retested in order to provide a negative control on the post-vaccination sera in the sensitive test. These sera comprised 6 with SR's of 6/6, 3/6, 2/6, and 1/6, and 30 of 0/6, the latter selected at random.

The interpretation of "inconclusive" results obtained in the sensitive tests differs from that which is generally made. It is the usual practice to consider sera with SR's of 2/6, 3/6, 4/6, 3/8, 4/8 and 5/8 to be "not positive"; we have interpreted them as weak positives because we consider them to be not negative. The SR's corresponding to negative and strongly positive are shown in the headings of Table 1.

Other tests

At the CFI the majority of the sera were submitted also to neutralization tests with dengue 2 (strain Tr 1751), St Louis, Ilheus (Strain Laemmert), and Bussuquara (strain Ar 41922), following the technique described by Smithburn et al. (1954). In these tests the test dilution of the virus was made also in undiluted fresh normal guinea-pig serum, and at least 50 LD_{50} of the virus were used. The results

TABLE 1

RESULTS OF NT FOR YELLOW FEVER ON SERA FROM VACCINATED AND PRESUMABLY UNVACCINATED PERSONS FROM POUSO ALEGRE, BLED IN 1958

Sera tested	Remarks on neutralization tests used	Survival ratios (SR) in neutralization tests									Total
		negative			weak positive			strong positive			
		0/6 0/7 0/8	1/6 1/7 1/8	— 2/7 2/8	2/6 3/7 3/8	3/6 — 4/8	4/6 4/7 5/8	— 5/7 6/8	5/6 6/7 7/8	6/6 7/7 8/8	
Persons vaccinated in 1941	Standard NT done at OCI, Rio de Janeiro	10	6	0	14	9	10	0	16	43	108
	Final results after retesting sera in a sensitive NT done at CFI, Bogotá [a]	1	1	1	6	2	15	8	25	49	108
	Percentage (final)	3			21			76			100
Persons presumably unvaccinated	Standard NT done at OCI, Rio de Janeiro	72	3	0	1	1	0	0	0	1	78
	Final results after retesting sera in a sensitive NT done at CFI, Bogotá [b]	68	9	0	0	0	0	0	1	0	78
	Percentage (final)	99			0			1			100

[a] Sera retested: 39 with SR's of 0/6, 1/6, or 3/6; 7 with SR's of 6/6 in the standard test at the OCI.

[b] Sera retested: 30 with SR's of 0/6 (taken at random); 6 with SR's of 6/6, 3/6, 2/6 and 1/6 in the standard test at the OCI.

were interpreted according to the guide proposed by Sawyer & Lloyd (1931). Although strain Ar 41922, isolated in Colombia by Groot, Morales & Vidales (1961), shows some immunological differences from prototype Bussuquara, it is considered to be merely a strain of Bussuquara.

Furthermore the sera were examined in haemagglutination-inhibition (HI) tests with 3 yellow fever antigens, prepared from strains 17D, and the Brazilian JSS; and with antigens prepared from the Laemmert strain of Ilheus virus, from the Tr 1751 strain of dengue 2, and from an unspecified strain of St Louis virus supplied by the Rockefeller Foundation Virus Laboratories. In the HI tests, made following Clarke & Casal's (1958) method, 8 units of antigen were used, and non-specific inhibitors in the sera were removed with kaolin. The sera were tested in serial twofold dilutions commencing at 1 : 10. The titre of a serum was taken as the highest dilution giving complete inhibition of haemagglutination, and was recorded as the exponent of the power of 2 which, multiplied by 5, gave the denominator of the dilution. Thus, 1 equals a titre of 1 : 10, 2 a titre of 1 : 20 and so on.

RESULTS

In Table 1 the results of the neutralization tests on the sera collected in Pouso Alegre in 1958 are summarized. Of the 108 sera from persons vaccinated in 1941 only 3, or 3%, gave results interpreted as negative, while 82, or 76%, gave strongly positive results, and 23, or 21%, gave weakly positive results. As to details: of the 16 sera that gave negative results with the standard test, 13 became weakly or strongly positive in the sensitive test; in addition there was a shift from weak to strong positives.

The small percentage of negative results in the current study is made more significant by the highly negative results obtained on the group of 78 children from Pouso Alegre that was included as a negative control on the post-vaccination sera. These sera were taken only from children who were said by a responsable adult never to have been vaccinated against yellow fever. It may be noted that 77 of the 78 sera gave NT results that were interpreted as negative, while 1 gave a strongly positive result. The details of the 3 sera that gave weak or strong positive results in the original standard tests are discussed

below, but the point to be noted at the moment is that the results of the sensitive tests were obtained without any increase in the numbers of mice surviving in the tests with sera that are truly negative. To be stressed is the fact that no mice survived in tests of 68 sera, a single mouse in tests if 8 sera, and 2 mice in a test of 1 serum. It is hence obvious that the positive results in the sensitive tests are not due to the survival of mice because of failure to inoculate enough virus to kill all the mice, even in the absence of specific antibody.

The positive results in the children who were reported not to have been vaccinated require comment. When the results of all the neutralization tests were in hand, it was noted that serum No. Q.87 044 from MJF, aged 12 years, had given a 6/6 positive result in a severe test done in Río de Janeiro, and a 1/8 negative result in a sensitive test in Bogotá. The serum was retested in Bogota in another sensitive test with a virus dosage of 100 LD_{50}. Again a negative result with SR of 0/8 was obtained. A special visit to Pouso Alegre revealed that MJF had never left Pouso Alegre and had never been vaccinated. On the other hand, careful inquiry about another child, LHF aged 10 years, whose serum was given the number Q.87 004, revealed that she had in fact been vaccinated against yellow fever in the town of Itajubá in 1952. Serum No. Q.87 004 gave negative results in both Rio de Janeiro and Bogotá.

It would appear probable that a mix-up between the two sera bearing the numbers Q.87 004 and Q.87 044 occurred in the Rio Laboratory. But we are at a loss to explain why neither of the sera gave positive results in the sensitive tests in Bogotá. Moreover, both sera gave negative results in HI tests with 3 yellow fever antigens, and with Ilheus, dengue-2 and St Louis antigens; and also negative results by NT with Ilheus, dengue 2 and St Louis viruses.

Serum No. Q.87 008 gave a 3/6 result in Rio and a 7/8 result in Bogotá, precisely as would be expected if it contained a small amount of yellow fever neutralizing antibody. Inquiry revealed that the 12-year old boy, JMO, from whom the serum was taken, had been vaccinated against some disease, possibly yellow fever, in Itajubá, a few years previously. Serum Q.87 008 did not exhibit HI antibodies.

Finally, there is the serum No. Q.86 996 which gave a 2/6 result in Rio, and an 0/8 result in Bogotá, and did not show HI antibodies. No field investigation was made, nor were special inquiries made about the vaccination history of its donor, and the Bogotá result is accepted as the final one. In all three cases the results obtained in Bogotá have been accepted as final because by so doing the least positive results have been used in interpreting the findings.

The results of the current study are similar to those obtained by Fox, Da Cunha & Kossobudzki (1948) on persons vaccinated with 17D vaccine in 1937 at Varginha, Minas Gerais, Brazil, who were bled six years later. Of 78 sera from vaccinated persons at Varginha tested in the young mouse test

TABLE 2

DISTRIBUTION OF RESULTS OF NEUTRALIZATION TESTS FOR YELLOW FEVER ON POST-VACCINATION SERA BY AGE OF DONOR

Age in 1941 when vaccinated (years)	Age in 1958 when bled (years)	Number				Percentage			
		negative	positive weak	positive strong	total	negative	positive weak	positive strong	total
1-9	18-27	0	5	16	21	0	24	76	100
10-19	28-37	0	3	28	31	0	10	90	100
20-29	38-47	1	4	22	27	4	15	81	100
30-39	48-57	2	7	7	16	12	44	44	100
40-49	58-67	0	3	7	10	0	30	70	100
50 +	68 +	0	1	2	3	0	33	67	100
	Total	3	23	82	108	3	21	76	100

described by the authors, 16 gave negative results (SR's of 0/6 and 1/6). However, when these 16 sera were re-examined in a sensitive young mouse test, only one (1%) gave a result which was interpreted as negative. Thirteen sera (17%) gave results interpreted as weak positive, and 63 (81%) gave strongly positive results. The similarity of the results is apparent in spite of the fact that no comparison of the sensitivities of the tests used has been made.

An analysis of the combined results of standard and sensitive tests reveals no suggestion of increasing positivity with increasing age, as shown in Table 2. This is additional evidence of the absence from the area not only of yellow fever virus but of any other virus that stimulated the presence of yellow fever neutralizing antibody.

It was possible to test by NT 90 sera from the group of vaccinated, and 68 from the group of presumably unvaccinated, persons, with dengue 2, Ilheus and St Louis viruses. Moreover, 68 sera were tested by NT with Bussuquara. The results, presented in Table 3, show that 6 persons (7%) in the vaccinated group gave positive results with yellow

TABLE 3

RESULTS OF NT AND HI TESTS ON SERA FROM VACCINATED AND PRESUMABLY UNVACCINATED PERSONS FROM POUSO ALEGRE BLED IN 1958

Sera tested	Results of NT with YF virus	Number of sera	Results of NT with viruses [a]				HI tests: number [b] of sera giving positive results with antigens					
			Dengue 2	Ilheus	St Louis	Bussu-quara	YF 17D	YF JSS	YF FN	Ilheus	Dengue 2	St Louis
90 persons vaccinated in 1941	69 strong positive	1	P	N	P	P	—	—	—	—	—	—
		1	P	N	P	N	—	—	0	1	—	0
		1	P	N	N	N	—	—	0	0	1	0
		1	P	N	N	—	1	1	1	1	1	1
		1	I	I	P	N	0	0	0	1	0	1
		1	I	N	P	N	—	—	0	0	0	0
		1	I	N	I	N	1	1	1	1	1	1
		1	I	N	I	—	—	—	0	0	0	0
		3	I	N	N	N	1/1	1/1	1/3	3/3	1/3	1/3
		2	I	N	N	—	2	2	2	2	1	2
		3	N	N	I	—	0/2	0/2	0/2	2/3	0/3	2/3
		18	N	N	N	N	8/16	8/17	3/6	4/18	3/18	5/18
		35	N	N	N	—	16/27	9/28	8/23	3/34	2/35	4/35
	18 weak positive	1	I	N	N	N	1	0	0	1	0	1
		8	N	N	N	N	3/8	4/8	1/1	1/8	1/8	1/8
		9	N	N	N	—	3/7	2/9	0/5	1/9	1/9	1/9
	3 negative	1	I	N	N	N	1	1	1	1	0	1
		2	N	N	N	N	0	0	0	0	0	0
68 persons presumably unvaccinated	68 negative	1	P	P	N	P	1	1	1	1	1	1
		1	I	N	P	I	0	0	1	1	1	1
		1	I	N	N	—	0	0	0	0	0	0
		39	N	N	N	—	1	0	0/30	0	0	1
		26	N	N	N	N	0	0	0/2	0	0	0

[a] Each serum was tested by NT with at least 4 viruses; P = positive; I = inconclusive; N = negative; — = not done.

[b] When not all the sera in each group were tested, the result is expressed as the ratio number positive/number tested.

fever and with one or more of the other Group B viruses tested. In the group of unvaccinated persons, 2 sera (3%) gave positive results: 1 for St Louis, and 1 for Ilheus, dengue 2 and Bussuquara.

The analysis by age of the results of the neutralization tests with dengue 2, St Louis and Ilheus, presented in Table 4, and showing no regular increasing positivity with increasing age, is suggestive also of the absence from the area of the aforementioned viruses. It is believed, therefore, that the antibodies for dengue 2, Ilheus, St Louis, and Bussuquara were acquired in some other place, rather than in Pouso Alegre. The incidence in persons over 50 years of age is understandable since older people have had more opportunities for travelling.

The presence of multiple positives ascertained by NT makes it difficult to define the agent or agents responsible for the antibodies. Among the group of vaccinated persons, the first 2 cases in Table 3 (one quadruple positive and one triple positive) indicate merely double or multiple exposure to Casal's Group B agents, one of which was indeed 17D. Then there are 2 cases positive by NT for yellow fever and dengue 2, of which in HI tests one showed positivity only to dengue 2, and the other a broad response to all antigens with higher titres for Ilheus and St Louis. A past infection with dengue 2 is therefore possible in both instances. Of 2 cases positive for yellow fever and St Louis and inconclusive for dengue 2, one showed HI antibodies to St Louis and Ilheus. These results are compatible with a past St Louis infection, in addition, of course, to the yellow fever vaccination. Among the unvaccinated individuals, one exhibited a triple positivity by NT and a corresponding broad response in HI tests; the other—positive for St Louis by NT, and exhibiting the highest titre of HI antibodies also for St Louis—suggests a past infection with this or a closely related virus.

The significance of the finding of 11 sera with neutralizing antibody for yellow fever, and giving inconclusive results by NT with dengue 2 and/or St Louis, is not clearly understood. Their results in HI tests are the following: in 1 case (inconclusive for dengue 2 and St Louis) negative result; in 1 case (inconclusive for dengue 2) antibodies in identical titres to yellow fever Ilheus and St Louis antigens;

TABLE 4

RESULTS OF 158 SERA TESTED BY NT WITH ILHEUS, ST LOUIS AND DENGUE 2 VIRUSES: VACCINATED AND PRESUMABLY UNVACCINATED PERSONS FROM POUSO ALEGRE BLED IN 1958, ANALYSED BY AGE

Sera tested	Age-group in 1958 (years)	Number examined	Number positive [a]	Percentage positive	Number positive for		
					Dengue 2	Ilheus	St Louis
68 persons presumably unvaccinated	5-9	19	0	0	0	0	0
	10-14	44	2 [b]	5	1	1	1
	15-19	5	0	0	0	0	0
90 persons vaccinated in 1941	15-19	2	0	0	0	0	0
	20-29	26	2 [c]	8	1	0	2
	30-39	19	0	0	0	0	0
	40-49	18	0	0	0	0	0
	50-59	15	3 [d]	20	3	0	1
	60+	10	1 [e]	10	0	0	1
	Total	158 [f]	8	5	5	1	5

[a] Number of positives for one or more of the three viruses.
[b] One for Ilheus, dengue 2 and Bussuquara; one for St Louis.
[c] One for St Louis, dengue 2 and Bussuquara; one for St Louis.
[d] Two for dengue 2; one for dengue 2 and St Louis.
[e] For St Louis.
[f] 68 of these sera were also tested by NT with Bussuquara.

in 3 cases (2 inconclusive for dengue 2 only, and 1 inconclusive for both dengue 2 and St Louis) antibody titres to yellow fever higher than those to heterologous antigens; and, finally 6 cases (3 inconclusive for dengue 2 and 3 for St Louis) in which the highest titres were observed with Ilheus and/or St Louis antigens. These last 6 cases suggest past exposure to some Group B agent besides 17D, perhaps that one for which the inconclusive results were obtained. For the 3 cases showing HI antibody in higher titres for yellow fever no satisfactory explanation can be offered. The HI pattern in these 3 individuals, aged 32, 57 and 62 years, does not suggest a past double exposure (i.e., 17D and dengue 2), at least a recent one; however, an old double exposure cannot be ruled out entirely because it is not known how long after a second infection the high and widely overlapping HI titres persist. On the

TABLE 5

SUMMARY OF RESULTS OF HI TESTS ON SERA FROM VACCINATED AND PRESUMABLY UNVACCINATED PERSONS FROM POUSO ALEGRE BLED IN 1958

Sera tested	Results of NT with yellow fever	Results [a] of NT with Ilheus, dengue 2 and St Louis	Results of HI tests with 8 units of antigens					
			YF 17D	YF JSS	YF FN	Ilheus	dengue 2	St Louis
Persons vaccinated in 1941	strong positive	NC	9/11 [b]	5/11	6/9	2/11	2/11	2/11
		P-I	5/8	5/8	5/14	11/15	5/14	8/15
		negative	24/43	17/45	11/29	7/52	5/53	9/53
	weak positive	NC	1/4	0/4	1/4	0/5	0/4	0/4
		P-I	1/1	0/1	0/1	1/1	0/1	1/1
		negative	6/15	6/17	1/6	2/17	2/17	2/17
	negative	P-I	1/1	1/1	1/1	1/1	0/1	1/1
		negative	0/2	0/2	0/2	0/2	0/2	0/2
	total		47/85	34/89	25/66	24/104	14/103	23/104
	percentage [c]		55	38	38	23	14	22
	all	total P-I	7/10	6/10	6/16	13/17	5/16	10/17
		%	70	60	38	77	31	59
	all	total negative	30/60	23/64	12/37	9/71	7/72	11/72
		%	50	36	32	13	10	15
Persons presumably unvaccinated	positive	NC	0/1	0/1	0/1	0/1	0/1	0/1
	negative	NC	0/8	0/8	0/7	1/9	0/9	1/9
		P-I	1/3	1/3	2/3	2/3	2/3	2/3
		negative	1/65	0/65	0/32	0/65	0/65	1/65
	total		2/77	1/77	2/43	3/78	2/78	4/78
	percentage		3	1	5	4	3	5

[a] NC = Incomplete (not tested with all 3 viruses); P-I = positive or inconclusive with one or more of the 3 viruses; Neg = negative with all 3 viruses.

[b] Ratio of number positive/number examined.

[c] Percentage of positive sera in HI tests.

TABLE 6

RESULTS OF HI TESTS ON 60 SERA FROM VACCINATED PERSONS WHO DID NOT EXHIBIT NEUTRALIZING ANTIBODIES TO ILHEUS, DENGUE-2 AND ST LOUIS VIRUSES

Results of NT for yellow fever	Number of sera	Average titres of sera giving positive results with 8 units of antigens				
		YF 17D	YF JSS	Ilheus	Dengue 2	St Louis
2 negative	2	N[a]	N	N	N	N
15 weak positive	8	N	N	N	N	N
	1	N	1.0	N	N	N
	4	1.8	N	N	N	N
	1	1.0	1.0	N	N	N
	1	4.0	5.0	7.0	6.0	6.0
43 strong positive	18	N	N	N	N	N
	1	N	1.0	N	N	N
	8	1.8	N	N	N	N
	3	2.8	1.1	N	N	N
	1	3.0	1.0	N	N	1.0
	1	1.0	N	N	N	1.0
	6	1.5	1.5	3.3	1.5	3.0

[a] N = Negative.

other hand, it is worth mentioning that H. Groot & A. Gast (*Resultados de la vacunación con 17D por escarificación y por multipuntura* (unpublished report)) have shown the occurrence of occasional inconclusive and even positive neutralization tests for dengue 2 as the result of a recent vaccination or revaccination with 17D.

One of the 2 inconclusives for dengue 2, which showed no demonstrable neutralizing antibody for yellow fever, exhibited HI antibodies to yellow fever, Ilheus and St Louis, with higher titres for the two latter. A past infection with some group B agent is suspected in this case.

References to the HI results have already been made in an attempt to understand the meaning of the results of the neutralization tests. Now the full results of the HI tests, presented in Table 5, are discussed. They show that a significant proportion of the sera from vaccinated people exhibited HI antibodies, whereas the proportion was very low among the presumably unvaccinated persons. In the latter group, out of 65 individuals with no demonstrable neutralizing antibody to yellow fever, Ilheus, dengue 2 and St Louis, there was only 1 positive serum with the low titre of 1 (1 : 10) for both 17D and St Louis antigens. This specimen was not tested with Bussuquara. Of the 4 sera from unvaccinated persons showing neutralizing antibodies to yellow fever or other group B agents, 2 gave positive results in the HI tests.

Among the vaccinated individuals, it was possible to establish that 72 did not present neutralizing antibodies to dengue 2, Ilheus and St Louis. Furthermore, 28 of this group, when tested with Bussuquara by NT, also gave negative results. Sixty of the 72 sera were examined in HI tests with 8 units of the following antigens: 17D, JSS, Ilheus, dengue 2 and St Louis. The results of the HI tests, presented in Table 6, could be divided into two main categories: first, those which reacted with all antigens, and second, those which reacted with only some of the antigens or did not react with any of them.

Into the first category fall 7 specimens (1 weak positive and 6 strong positive for yellow fever by NT) all of which exhibit higher titres for Ilheus antigen. The HI pattern in these individuals (aged 22, 32, 40, 41, 44, 45 and 51 years) is suggestive of past exposure to some group B agent besides the vaccination with 17D 17 years before. Three of the

sera were not tested with Bussuquara; since the remaining 4, when tested by NT with Bussuquara, also gave negative results, it is likely that at least in 4 cases Ilheus, dengue 2, St Louis and Bussuquara have not played a role in the production of the immunological picture. The possible occurrence of a group B agent other than those mentioned is thus suspected. The possibility that the HI pattern in these cases is the result solely of the vaccination performed 17 years before is unlikely, since it has been demonstrated that in primary infections of yellow fever (Theiler & Casals, 1958) and after vaccination with 17D of persons never exposed to other group B agents (Groot & Gast, unpublished data) the titres of homologous HI antibodies are always as high as, or higher than, those of heterologous antibodies. The probable role of repeated vaccination is not discussed because adequate information is lacking.

Into the second category fall 53 sera which are either negative with all antigens (28) or positive with some (25). Twenty-three of the positive sera are positive only to yellow fever antigens; the remaining 2 are positive to yellow fever and St Louis antigens, but with titres to yellow fever as high as, or higher than, those to St Louis. These 53 sera indicate the HI antibody pattern 17 years after vaccination with 17D in persons with a negative serological evidence of exposure to Group B viruses other than yellow fever. The influence of possible revaccinations, however, cannot be ruled out. It is observed that 23 of the 53 sera (43%) exhibited antibodies for 17D, with an average titre of 2.1; and that 12 sera (23%) were positive for JSS, with an average titre of 1.1. These facts indicate that after vaccination with 17D not only does the proportion of sera exhibiting HI antibodies diminish with increasing time, but so also do the titres. As a point of reference it is worth mentioning that Groot & Gast (unpublished data) found that 92.8% of 263 persons showed HI antibodies for 17D antigen 30-40 days after vaccination with 17D by scarification or multipuncture. The average titre of the positive sera was 3.1. The proportion of positives for JSS in the same group was lower (32.1% of 190), as well as the average titre of the antibody for JSS: 1.5. The pre-vaccination sera in this group also showed negative evidence of exposure to Group B viruses.

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RÉSUMÉ

Dans une région du Brésil où la fièvre jaune n'a jamais été signalée, mais où des vaccinations antiamariles ont été effectuées avec le vaccin 17D sur quelque 5000 personnes, en 1940-41, les auteurs ont étudié en 1958 le niveau d'anticorps subsistant chez certaines des personnes vaccinées. Un total de 108 échantillons de sérums, provenant de personnes dont les noms figuraient sur les registres de vaccination, a été soumis aux tests de neutralisation et d'inhibition de l'hémagglutination, pour détermination de la teneur en anticorps antiamarils et en autres anticorps de virus du groupe B.

Les tests de neutralisation pour la fièvre jaune donnèrent 3% de négatifs, 76% de fortement positifs, et 21% de faiblement positifs. Le test d'hémagglutination donna des proportions variables de positifs. Parmi 53 vaccinés pour lesquels les tests sérologiques étaient négatifs pour les virus B autres que la fièvre jaune, 43% avaient un titre moyen d'anticorps de 2,1 vis-à-vis de l'antigène 17D, et 23% des anticorps inhibiteurs de l'hémagglutination au titre de 1,1, vis-à-vis de l'antigène de la souche amarile brésilienne JSS.

REFERENCES

Clarke, D. & Casals, J. (1958) *Amer. J. trop. Med. Hyg.*, **7**, 561

Fox, J. P. & Cabral, A. S. (1943) *Amer. J. Hyg.*, **37**, 93

Fox, J. P., Da Cunha, J. F. & Kossobudzki, S. L. (1948) *Amer. J. Hyg.*, **47**, 64

Fox, J. P., Kossobudzki, S. L. & Da Cunha, J. F. (1943) *Amer. J. Hyg.*, **38**, 113

Groot, H., Morales, A. & Vidales, H. (1961) *Amer. J. trop. Med. Hyg.*, **10**, 397

Sawyer, W. A. & Lloyd, W. (1931) *J. exp. Med.*, **54**, 533

Smithburn, K. C., Kerr, J. A. & Gatne, P. B. (1954) *J. Immunol.*, **72**, 248

Theiler, M. & Casals, J. (1958) *Amer. J. trop. Med. Hyg.*, **7**, 584