VACCINATION WITH VIRUS 17D IN THE CONTROL OF JUNGLE YELLOW FEVER IN BRAZIL

by **Fred L. Soper** and **H. H. Smith,** International Health Division of the Rockefeller Foundation, Rio de Janeiro

33

Reprinted from the Transactions of the Third International Congress of Tropical Medicine and Malaria, 1938, Vol. I, pp. 295-313

PRINTED IN HOLLAND BY C. A. SPIN & ZOON N.V.

VACCINATION WITH VIRUS 17D IN THE CONTROL OF JUNGLE YELLOW FEVER IN BRAZIL[1]

by **Fred L. Soper** and **H. H. Smith,** International Health Division of the Rockefeller Foundation, Rio de Janeiro

Vaccination in Jungle Yellow Fever

The first attempt to protect an exposed population against jungle yellow fever by vaccination was made in Paraná, Brazil, early in 1936 (1), using hyperimmune goat and monkey sera and a virus modified by culture in mouse embryo tissue (17E) (2). The difficulties encountered were such as to cause the discontinuation of this method in the field, and during the yellow fever season (January to May) of 1937, no attempt was made to protect exposed populations.

Work with another modified virus (17D) developed in the laboratories of the International Health Division of The Rockefeller Foundation in New York, began in Brazil in February, 1937 (3). By June, these studies had progressed far enough to justify field vaccination, and the county of Varginha, Minas Geraes, in a region where jungle yellow fever had been found a few weeks previously, was chosen for the first field application of the new vaccine virus. During the next three months, 2,746 persons were vaccinated in the field, with satisfactory results, and, in September, routine field vaccination began, which increased the total field vaccinations for the year 1937 to 36,104.

The 1938 yellow fever season in South Brazil began early in January, with an outbreak of jungle yellow fever at Presidente Wenceslau, São Paulo (4), and shortly thereafter the disease was found at Mathias Barboza, Minas Geraes. Vaccination units were moved into both these districts, and an attempt was made throughout the following months to vaccinate threatened populations wherever yellow fever was found. The 1938 yellow fever season was an active one, with outbreaks in some of the richest and most heavily populated agricultural districts of Brazil, in the states of Minas Geraes, Rio de Janeiro, São Paulo and Santa Catharina. The need for vaccine greatly exceeded the initial production capacity of the laboratory and the

[1] This report is based on work of many colleagues of the Cooperative Yellow Fever Service, jointly maintained by the Ministry of Education and Health of Brazil and the International Health Division of The Rockefeller Foundation. Special credit for the rapid expansion of vaccination in 1938 must go to the Brazilian Government, which furnished the necessary additional funds.

ability of the field service to apply it. The Brazilian Government opened a special credit of 2,000 contos, or approximately $ 100,000 UScy., to cover the cost of a program for the vaccination of at least one million persons during the year 1938.

From January first to July 31st, a total of 557,861 persons were vaccinated, and the final figures for the year will almost certainly exceed the preliminary estimate of one million.

Table I gives the number of persons vaccinated per month in Brazil, from September, 1937, to July, 1938, by states. Table II gives the distribution of the same persons by population groups.

Origin of Vaccine Virus 17D

In December, 1933, Lloyd transferred the Asibi strain to tissue culture containing mouse embryo tissue and monkey serum ; after 18 subcultures, a second transfer was made to a medium containing whole chick embryo tissue, from which, after 56 passages, it was transplanted to tissue culture containing chick embryo, from which the central nervous system had been removed. After 39 passages in this medium, without central nervous system tissue, this strain of virus, now known as 17D, was tested and found to have lost much of its viscero- and neurotropism, while still retaining the property of stimulating the production of antibodies (5). Virus 17D was first used for human inoculation on November 30, 1936, in New York (6), with material transferred 227 times in tissue culture since its last previous passage in an animal host. Subcultures used as source of vaccine in Brazil have ranged from the 205th to the 317th.

Results Obtained with Virus 17D

The points on which a method of vaccination for general use as a public health measure should be judged, may be grouped under the following headings :

a. Ease of manufacture of standard product.
b. Ease of application under field conditions.
c. Safety and comfort of persons vaccinated.
d. Safety of persons not vaccinated.
e. Antibody production.

A. *Ease of Manufacture of the Standard Product*

The titer of virus in tissue culture material is much below that obtained by growth in the developing chick embryo. The vaccine

virus is maintained in tissue culture free of central nervous system tissues, to avoid any possible reversion to type, but for the preparation of vaccine, tissue culture is inoculated in the allantoic sac of the six-day old chick embryo. After further incubation for four days, at a temperature of 37° C., the embryo is removed, triturated, and suspended (10%) in inactivated human serum diluted with equal amounts of distilled water. The filtrate of this suspension, which is the vaccine material, is distributed in ampoules, frozen, dried in vacuum, sealed and stored at about 2° C. In addition to the usual bacteriological controls for sterility, each lot of vaccine is titrated for virus content by intracerebral inoculation in serial dilutions in white mice, and is inoculated intracerebrally into a *rhesus* monkey, to test for possible increase in either viscero- or neurotropism.

Altough laboratory studies indicate (3) that a much smaller dose may be sufficient, between 350 and 800 MLD [1] for mice are now being allowed for each person vaccinated in Brazil. On this basis the Rio de Janeiro Laboratory is producing some 120,000 doses of vaccine per month, at a total cost, including overhead, excepting rent, of less than $ 3,000 UScy., or 2½ cents UScy., per dose.

B. *Ease of Application under Field Conditions*

Virus 17D, even when dried and sealed, is susceptible to ordinary temperatures and to direct sunlight; the vaccine leaves the Rio laboratory, packed with ice and salt, in wide-mouthed thermos flasks, and is thus kept chilled until the moment of rehydration. Even after rehydration with distilled water the ampoule is kept on ice, and the vaccine is finally diluted in physiological saline solution in the syringe itself immediately preceding inoculation. To determine the viability of the virus used, mice are inoculated intracerebrally with the remaining vaccine after the last person has been inoculated.

Experience shows that a vaccination unit, consisting of three persons, a doctor, a technical assistant and a secretary-chauffeur, can, under optimum conditions, register and inoculate from 1,000 to 2,000 persons a day [2]. The actual cost of applying vaccine in Brazil

[1] The end point of titration in mice is considered as that dilution which, when injected in 0.03 c.c. amounts intracerebrally in mice, will produce a mortality of 50% (7).

[2] The use of three Forsbeck needle-racks by each unit is advisable, to avoid unnecessary delays in waiting for needles to cool after boiling. It is believed that certain irregular results of postvaccination protection tests are due to failure to cool needles after boiling, with consequent inactivation of the vaccine virus.

in 1938 has not exceeded, including initial cost of automobiles and equipment, 7 cents UScy., *per capita*. The actual field operating expense has dropped from 5½ cents UScy., *per capita*, in January, to 3 cents UScy., in June. However, the *per capita* cost of application must increase rapidly in sparsely populated regions and in areas where transportation is difficult.

C. *Safety and Comfort of Persons Vaccinated*

Since the beginning of work with virus 17D in February, 1937, a conscientious search has been made among vaccinated groups for evidence of:

1. Severe reaction at site of inoculation;
2. Sensitization to foreign protein;
3. Serum sickness;
4. Virus reaction, visceral and neural;
5. Delayed jaundice, and
6. Infection with other viruses.

Special attention should be called to the distribution of vaccinated persons by population groups (Table II). Employees of the Yellow Fever Service, of the airlines, the population of large coffee fazendas, inmates of schools, laborers and highway construction gangs and members of military units, all form very useful groups for observation. Even where it has not been possible for physicians of the Yellow Fever Service to make personal observation, fazenda owners, military medical officers, school directors and other responsible persons have given information as to the severity of postvaccination reactions.

The sum total of observations on vaccinated groups may be stated briefly as follows:

For the eighteen months' period, during which almost 600,000 persons were vaccinated, there is no evidence of severe reaction at the site of inoculation, of sensitization to foreign protein [1], of serum sickness, of delayed jaundice (8), (9), nor of infection with other viruses.

The type of relatively mild reaction which is observed seems to be a general, not neural, reaction to the virus itself, after an

[1] A number of cases have received second and third inoculations of 17D, without any evidence of sensitization to chick protein.

incubation period of generally from five to eight days. [1]

The symptoms most frequently noted are : headache, backache, body pains, weakness and malaise, lasting from a few hours to a couple of days. The reaction to virus 17D is not severe enough to have any influence against its general acceptance by the people. Fazenda owners, and others responsible for large groups, generally report from 5 to 8% of reactions, with not more than 1 to 2% of reactions severe enough to cause loss of time from work. A person-to-person canvas, however, will result in 20, 40 or even 50% of individuals questioned reporting at least a slight headache, but the number of severe reactions does not increase correspondingly. The most severe reactions reported are those related to each other by members of the foreign colony in the capital city of Rio de Janeiro !

Considering the number vaccinated, it seems truly remarkable that many more conditions occurring after vaccination have not been credited to the inoculation. Experience has failed to reveal any contraindications to the use of virus 17D, early restrictions have been entirely removed, and children of all ages and women in all stages of pregnancy are routinely inoculated.

D. *Safety of Persons Not Vaccinated*

In using a living virus for vaccination, the possibility of such living virus being picked up from the blood stream by some insect vector, and sooner or later reverting to its original virulence, must be considered. Such return to virulence of a yellow fever vaccine would have to depend upon the following factors :

1. Circulation of virus in the blood stream in quantities sufficient to infect the insect vector ;
2. Ability of the infected vector to transmit the vaccine virus, and
3. Ability of the vaccine virus to revert to a virulent state.

Experimental work indicates that sufficient virus does not circulate to infect the traditional vector, *Aëdes aegypti*, and that even when this mosquito has been infected by special methods it does

[1] So far, only one case has been reported, in which symptoms of involvement of the central nervous system were attributed by the attending physicians to inoculation with virus 17D. Case E. R. C., observed by Drs. Raul Azevedo and Deolindo Couto, Rio de Janeiro, to whom we owe thanks for details of this case, developed signs of meningeal involvement one month after vaccination with Lot 136 of virus 17D, the estimated virus used being 220 MLD for mice. Complete recovery occurred, and studies are now in progress to determine, if possible, the nature of the infection.

not readily transmit the 17D virus, even after prolonged incubation (10). Attempts to infect *Aëdes aegypti* by postvaccination feeding on humans and on *rhesus* monkeys, which have been shown to circulate more virus than do humans, were failures, no virus being demonstrated in the mosquito by either feeding on monkeys or inoculation into mice. The immersion of *Aëdes aegypti* larvae in high concentration of virus did result in the production of infected mosquitoes, as demonstrated by mouse inoculation; such infected *aegypti* failed completely to transmit virus to susceptible animals, even after prolonged incubation periods.

The difficulty of getting virus 17D to circulate in appreciable quantities with regularity, has, so far, prevented conclusive experiments with the jungle vectors of yellow fever, only a few of which have very recently been definitely incriminated (11). The same difficulty has prevented the carrying out of a large series of animal passages, to determine the ability of virus 17D to revert to its original type; the relative stability of the virus in tissue culture, embryo passage and in mouse brain passage, suggests that such reversion to virulence, if it did occur at all, would be slow in appearing. This opinion is strengthened by the results of other workers, who have not been able to transmit a tissue culture virus with *Aëdes aegypti* (12), nor to reconvert it to virulence by direct liver-to-liver passage (13).

E. *Antibody Production*

The *rhesus* monkey, which is more highly susceptible to yellow fever than is man, becomes fully resistant to virulent strains, such as Asibi, following inoculation with virus 17D. Similar tests on humans have not been made, but the wide use of virus 17D this year, among exposed populations, during active outbreaks of jungle yellow fever, has resulted in a mass of field observation almost as conclusive as laboratory experiments. Local physicians and other observers report a sudden reduction in observed cases in infected districts shortly after mass vaccination, and cite instances in which individuals, who failed to be inoculated, later contracted the disease in infected forests, while vaccinated members of the same labor gangs escaped. Field experience suggests that the protective effect of vaccination begins not later than a week after inoculation, although laboratory tests fail to show demonstrable antibodies at this time (3). While it is probable that a much larger number of cases of yellow

fever must have occurred among persons infected before vaccination, only eight of these have been reported, four in Minas Geraes, three in Santa Catharina and one in São Paulo. Onset in two was on the same day as vaccination, in the other four, between the first and fourth days following. Two of the three fatal cases in this group were confirmed by viscerotomy, and a virus, quite different from the vaccine virus, was isolated from one of the non-fatal cases.

Two additional cases of postvaccination yellow fever have been found, one mild case with onset thirty days, and one fatal case with onset six weeks after vaccination. These cases had received virus from lots 95 and 117, both of which gave irregular results, as measured by the protection test (Table IV). It is possible that neither received active virus.

The mouse protection test (14) has been used since 1931, for determining the presence of yellow fever antibodies in the blood serum of persons and animals. It is customary to inoculate six mice with highly neurotropic virus and with the serum to be tested. Results are read as a fraction showing the proportion of mice living on the fourth day (denominator), which survive to the tenth day (numerator) after inoculation.

Seven readings are possible, of which only two, 6/6 and 5/6, are, in analyzing critical immunity surveys, considered definite evidence of previous infection with yellow fever; 4/6 and 3/6 results are considered inconclusive, and 2/6, 1/6 and 0/6 as negatives. It has been noted in immunity surveys that bloods from regions where yellow fever has never been present give remarkably clearcut negative readings, whereas bloods from endemic regions give an appreciable number of inconclusives, as well as positives and negatives. The majority of these inconclusives are probably from individuals who have at some time been exposed to yellow fever infection, and are, almost certainly, not apt to ever again develop clinical yellow fever. It seems reasonable at the present time to read mouse protection test results as indicating full protection, partial protection and no protection, without attempting to interpret too rigidly these readings in terms of reaction to yellow fever infection, further than to assume that those showing full protection are, at the moment tested, adequately protected against fully virulent virus. Postvaccination results, when compared with prevaccination results (Table III), suggest that virus 17D does produce some measurable antibody formation in almost 100% of persons receiving 50 MLD or more

of living virus. It has been noted that in many postvaccination protection tests, in which the final reading is: 2/6, 1/6, or even 0/6, the average length of survival of inoculated animals is from one to two days longer than for similar negative tests in vaccinated groups. This suggests that sufficient antibody is present to definitely delay the action of virus inoculated in animals.

Table III gives the results of pre- and postvaccination tests on the same individuals, including both laboratory and field groups [1], during the preliminary phase of observation, before routine field vaccination began. Attention must be called to the fact that on one occasion, the virus was apparently inactive before inoculation began, since all of the persons tested failed to give evidence of antibody development, and the inoculation of the remaining vaccine into mice failed to cause any deaths.

Table IV covers a special investigation to determine the results obtained with different dosages of virus, and to evaluate the viability test as an indication of efficiency of the preceding vaccination. The groups bled for this special study were selected as probably representative of the poorest work of the season, and included groups receiving the lowest doses of virus used during the height of the yellow fever outbreak, working with newly trained personnel, far from headquarters. The results indicate that doses as low as 50, 85 and 100 MLD per person are adequate to give satisfactory results. They also indicate that the viability test, in and of itself, is not a safe indication of the efficiency of the vaccination. For example, lot 117 of virus 17D was used and tested in five groups, of which only one gave satisfactory results, the viability tests for which, 0/5, 1/5 and 1/4, were poor. Postvaccination mouse protection tests on a number of persons from vaccinated groups are proving a better method of checking the work of field units than is the test for viability of the remaining vaccine.

Table V gives a general summary of all postvaccination protection test results for work with virus 17D in Brazil.

A study of Tables III, IV and V and other available information suggest that the differences in the results of vaccination depend in great part upon the delivery of relatively small amounts of active virus below the skin of the individual vaccinated. The results show

[1] Vaccination of these groups was carried out under the direct supervision of Dr. H. H. Smith, who, with Drs. Henrique Penna and Adhemar Paoliello (3), has published a report covering observations on the first 60,000 vaccinations in Brazil.

that with standardized methods of vaccine production and with adequate supervision of the administration of virus in the field, highly satisfactory results can be obtained.

Anticipated Epidemiological Results of Vaccination

Admitting that the individual can be protected by vaccination, the epidemiological results of vaccination must vary with the conditions under which infection occurs. Where man is an essential element in the cycle of infection, responsible for maintaining the virus, as in urban *aegypti*-transmitted yellow fever, artificial immunization of the bulk of the population should effectively protect the remaining non-immunes. It is probable that occasional mass vaccination will be found more economical and practicable in certain regions, for breaking the cycle of infection, man-*aegypti*-man, than is the traditional maintenance of antimosquito services for the prevention of *aegypti* breeding.

In considering jungle yellow fever, however, in which man is, apparently, not an important factor in maintaining the virus, vaccination should alter the epidemiological picture, mostly by preventing the infection of vaccinated persons, and, only in a very minor degree, by reduction of the source of virus for forest vectors.

Vaccination promises to be a great aid in preventing the transfer of yellow fever infection from one place to another by the human host; the long-distance transfer of virus, by modern methods of rapid transportation, can be prevented by vaccination, as can also the introduction of virus from jungle to urban areas. Since the jungle infection, apparently, exists independent of the human population, and spreads from place to place by other than human carriers, vaccination cannot be expected to completely eradicate yellow fever.

Summary.

During the period, September 1937 to July 1938, over half a million persons were inoculated with the modified yellow fever virus 17D. Vaccination with this virus was widely used throughout the 1938 epidemic of jungle yellow fever in South Brazil. Field observations indicate that vaccination becomes effective within a week after inoculation. Reaction to vaccination is relatively mild, and no contraindications have been found. The results of approximately 3,000 mouse protection tests are presented, showing that a high percentage of persons vaccinated develop demonstrable antibodies.

Bibliography

1. Soper, F. L. Vacinação contra a febre amarella no Brasil, de 1930 á 1937. *Arch. de Hig.* Rio de Janeiro. 1937. *7 :* 379-390.
2. Lloyd, Wray, Theiler, M., and Ricci, N. I. Modification of the virulence of yellow fever virus by cultivation in tissue *in vitro*. *Tran. Roy. Soc. Trop. Med. Hyg.* 1936. *29 :* 481-529.
3. Smith, H. H., Penna, H. A., and Paoliello, A. Yellow fever vaccination with cultured virus (17D) without immune serum. *Am. Jl. Trop. Med.* 1938. *In Press.*
4. Aragão, H. de B. Observações a respeito de um foco limitado de febre amarella sylvestre no Estado de São Paulo. *Brasil Medico.* Rio de Janeiro. 1938. *52 :* 401-412.
5. Theiler, M., and Smith, H. H. The effect of prolonged cultivation *in vitro* upon the pathogenicity of yellow fever virus. *Jl. Exp. Med.* 1937. *65 :* 767-786.
6. Sawyer, W. A. Experience in vaccinating against yellow fever with immune human serum and virus fixed for mice. *Am. Jl. Hyg.* 1937. *25 :* 221-231.
7. Reed, L. J., and Muench, H. A simple method of estimating fifty per cent endpoints. *Am. Jl. Hyg.* 1938. *27 :* 493-497.
8. Findlay, G. M., and MacCallum, F. O. Note on acute hepatitis and yellow fever immunization. *Trans. Roy. Soc. Trop. Med. Hyg.* 1937. *31 :* 297-308.
9. Soper, F. L., and Smith, H. H. Yellow fever vaccination with cultivated virus and immune and hyperimmune serum. *Am. Jl. Trop. Med.* 1938. *18 :* 111-134.
10. Whitman, L. Failure of *Aëdes aegypti* to transmit yellow fever cultured virus (17D). 1938. *In Press.*
11. Shannon, R. C., Whitman, L., and Franca, M. Yellow fever virus in jungle mosquitoes. *Science.* 1938. *88 :* (No. 2274) 110-111.
12. Roubaud, E., Stefanopoulo, G. J., and Findlay, G. M. Essais de transmission par les stégomyies du virus amaril de cultures en tissu embryonnaire. *Bull. Soc. Path. Exot.* 1937. *30 :* 581-583.
13. Findlay, G. M., and MacCallum, F. O. Vaccination contre la fièvre jaune au moyen du virus pantrope atténué employé seul. *Bull. Off. Internat. d'Hyg. Publ.* 1937. *29 :* 1145-1149.
14. Sawyer, W. A., and Lloyd, Wray. The use of mice in tests of immunity against yellow fever. *Jl. Exp. Med.* 1931. *54 :* 533-555.

TABLE I

Persons in Brazil vaccinated with virus 17D from September 1937 to July 31st 1938, away from the laboratory

Months	Federal district	Rio de Janeiro State	São Paulo State	Minas Geraes State	Santa Catarina State	Mato Grosso State	Total
September				3.759			3.759
October				10.580			10.580
November				7.473			7.473
December				11.540		6	11.546
January	46		8.103	12.701			20.850
February	3.337	1.861		31.557		11	36.766
March	13.455	18.234		64.558			96.247
April	10.313	17.238		45.084	22.363		94.998
May	6.181	12.894		66.340	12.005	11	97.431
June	5.224	16.760	5.406	72.393			99.783
July	6.444	10.726	11.259	83.357			111.786
Total	45.000	77.713	24.768	409.342	34.368	28	591.219

TABLE II

Distribution by population groups of persons vaccinated in Brazil

September 1937 to July 31st 1938

Population group	September to December 1937	January to July 1938	Total
Farms and hamlets	16.530	397.809	414.339
Military units	1.105	23.730	24.835
Schools	994	34.348	35.342
Labor gangs	368	39.183	39.551
Cities and towns	14.361	53.337	67.698
Miscellaneous	—	9.454	9.454
Total	33.358	557.861	591.219

TABLE III Immunity to yellow fever following vaccination with Virus 17D measured by mouse protection test

Lot No.	Dilution	Dose M.L.D[1] (Mouse)	Where used	Viability test result[2]	Persons inoculated	Number tested	Mouse protection test results[3] 0/4 0/5 0/6	1/4 1/5 1/6	2/5 2/6	2/4 3/6	3/5 4/6	3/4 4/5 5/6	4/4 5/5 6/6	% of mice surviving	Protection index[4]
39	1 : 1)	850 to	Laboratory	5/6,6/6	20	18									
	1 : 2)	7.500		Pre-vaccination			14	2	0	0	1	1	0	10	0.6
				Post-vaccination			0	0	0	0	3	4	11	89	5.4
40	1 : 1)	85.000	Laboratory	26/26	71	52									
	1 : 2)			Pre-vaccination			43	6	0	0	0	1	2	8	0.4
				Post-vaccination			1	0	0	5	7	11	28	82	5.1
41	1 : 2	25.000	Field	18/18	77	66									
				Pre-vaccination			54	9	2	0	0	0	1	5	0.3
				Post-vaccination			0	0	3	3	18	22	20	78	4.8
41	1 : 2)	25.000	Laboratory	23/23		10									
	und)			Pre-vaccination			4	4	0	0	0	1	1	26	1.5
				Post-vaccination			0	0	0	0	0	2	8	96	5.8
42	1 : 2	11.000	Field	23/23	141	132									
				Pre-vaccination			109	16	3	0	0	2	2	5	0.3
				Post-vaccination			0	0	0	7	25	42	58	85	5.1
52	1 : 1	25.000	Field	23/23	79	69									
				Pre-vaccination			54	13	0	1	0	0	1	5	0.3
				Post-vaccination			0	0	0	1	15	29	24	84	5.1
52	1 : 10	2.500	Field	27/27,1/2	589	159									
				Pre-vaccination			122	26	8	2	0	0	1	4	0.3
				Post-vaccination			4	1	2	18	46	54	34	76	4.5
52	1 : 10	2.500	Field	0/10,0/7	172	21									
				Pre-vaccination			17	2	0	0	0	0	2	11	0.7
				Post-vaccination			15	4	0	0	0	0	2	13	0.8
				TOTAL	1149	527									
				Pre-vaccination			417	78	13	3	1	5	10	6	0.4
				Post-vaccination			20	5	5	34	114	164	185	78	4.7

[1] The endpoint for titration of virus in mice is calculated on the basis of 50 % mortality.

[2] The fraction indicates the number of *mice dying of specific encephalitis* (numerator) in comparison with number alive four days after inoculation (denominator).

[3] The fraction indicates the number of *mice surviving to the tenth day* (numerator) in comparison with number alive four days after inoculation (denominator).

[4] Average number of mice surviving calculated on basis of six mouse groups.

TABLE IV

Immunity to yellow fever following vaccination with Virus 17D measured by mouse protection test

Lot No.	Dilution	Dose M.L.D[1] (Mouse)	Where used	Viability test result[2]	Persons inoculated	Number tested	Mouse protection test results[3] 0/4 0/5 0/6	1/4 1/5 1/6	2/5 2/6	2/4 3/6	3/5 4/6	3/4 4/5 5/6	4/4 5/5 6/6	% of mice surviving	Protection index[4]
69	1 : 5	1500	Farms & Hamlets	0/6	47	10	0	1	0	0	1	1	7	88	5.2
79	1 : 5	85	Cit. & Towns	1/6	338	17	2	0	0	1	3	3	8	81	4.6
	1 : 5	85	Milit. Units	6/6	143	20	0	0	0	0	0	4	16	96	5.8
80	1 : 5	130	Farms & Hamlets	3/5	329	21	1	0	1	0	1	7	11	84	5.1
	1 : 5	130	Farms & Hamlets	1/5	350	23	1	0	1	1	2	7	11	82	5.0
85	1 : 5	50	Cit. & Towns	2/6	364	21	3	0	0	0	2	6	10	77	4.7
	1 : 5	50	Cit. & Towns	6/6	245	20	6	0	0	1	0	5	8	63	3.8
95	1 : 20	850	Farms & Hamlets	1/6	237	21	10	3	2	0	1	0	5	32	2.0
	1 : 20	850	Farms & Hamlets	4/4	476	20	1	0	0	1	1	3	14	87	5.3
101	1 : 20	85	Schools	2/5	72	12	0	0	0	0	1	0	11	97	5.8
	1 : 20	85	Farms & Hamlets	6/6	108	10	0	0	0	0	1	4	5	89	5.4
	1 : 10	170	Farms & Hamlets	18/18	195	21	0	0	0	0	5	3	13	89	5.4
102	1 : 20	270	Cit. & Towns	3/6	94	22	0	0	0	0	2	4	16	96	5.6
	1 : 20	270	Farms & Hamlets	6/6	353	20	0	0	0	1	3	5	11	88	5.3
	1 : 10	540	Farms & Hamlets	16/16	111	20	1	0	0	1	3	1	14	87	5.2
	1 : 20	270	Cit. & Towns	6/6	11	11	0	0	1	0	0	3	7	90	5.4
103	1 : 10	100	Farms & Hamlets	4/6	399	20	0	0	0	0	4	5	11	89	5.4
	1 : 10	100	Farms & Hamlets	6/6	414	20	0	0	0	0	1	6	13	93	5.6
106	1 : 5	85	Farms & Hamlets	4/6	269	20	3	0	0	0	1	7	9	77	4.7
	1 : 5	85	Cit. & Towns	4/5	353	21	10	1	1	1	0	4	4	41	2.4
115	1 : 10	110	Farms & Hamlets	0/4	417	21	1	0	0	3	1	6	10	88	4.9
	1 : 10	110	Schools	0/4	634	21	0	0	0	0	3	7	11	89	5.4
	1 : 10	110	Farms & Hamlets	5/5	119	19	2	0	0	1	1	7	8	78	4.7
	1 : 10	110	Farms & Hamlets	5/5	109	21	1	1	1	1	0	5	12	82	4.9
117	1 : 20	85	Farms & Hamlets	0/5	405	5	0	0	0	0	0	1	4	96	5.8
	1 : 20	85	Farms & Hamlets	1/5	192	11	0	1	0	0	0	5	5	84	5.1
	1 : 20	85	Cit. & Towns	6/6	356	21	1	0	0	0	3	5	12	86	5.2
	1 : 20	85	Cit. & Towns	6/6	243	20	5	1	0	0	2	5	7	64	3.8
	1 : 10	170	Farms & Hamlets	5/5	60	20	3	2	0	0	2	6	7	68	4.1
	1 : 20	85	Farms & Hamlets	1/6	384	21	6	1	0	1	0	7	6	60	3.6
	1 : 20	85	Farms & Hamlets	4/6,10/12, 5/5	1201	54	3	3	5	7	6	12	18	70	4.2
126	1 : 20	200	Farms & Hamlets	3/4		42	7	1	3	0	1	12	18	71	4.3
	1 : 20	200	Farms & Hamlets	0/5	1307	36	6	1	1	0	3	7	18	73	4.4
	1 : 20	200	Farms & Hamlets	1/5		42	11	0	2	0	5	10	14	63	3.8
137	1 : 20	280	Cit. & Towns	4/4	954	39	1	1	0	1	3	17	15	83	5.0

The endpoint for titration of virus in mice is calculated on the basis of 50 % mortality.
The fraction indicates the number of *mice dying of specific encephalitis* (numerator) in comparison with number alive four days after inoculation (denominator).
The fraction indicates the number of *mice surviving to the tenth day* (numerator) in comparison with number alive four days after inoculation (denominator).
Average number of mice surviving calculated on basis of six mouse groups.

TABLE V

Immunity to yellow fever following vaccination with Virus 17D measured by mouse protection test

Lot No.	Dilution	Dose M.L.D[1] (Mouse)	Where used	Viability test result[2]	Persons inoculated	Number tested	Mouse protection test results [3] 0/4 0/5 0/6	1/4 1/5 1/6	2/5 2/6	2/4 3/6	3/5 4/6	3/4 4/5 5/6	4/4 5/5 6/6	% of mice surviving	Protection index[4]
39	1 : 1) 1 : 2)	850 to 7.500	Laboratory	63/65	20	18	0	0	0	0	3	4	11	89	5.4
40	1 : 1) 1 : 2)	85.000	Laboratory	201/202	71	52	1	0	0	5	7	11	28	82	5.1
41	1 : 2	25.000	Field and	18/18	77)	66	0	0	3	3	18	22	20	78	4.8
41	1 : 2	25.000	Laboratory	27/29	)	10	0	0	0	0	0	2	8	96	5.8
42	1 : 2	11.000	Field	34/34	141	132	0	0	0	7	25	42	58	85	5.1
52	1 : 1	25.000	Field	23/23	)	69	0	0	0	1	15	29	24	84	5.1
52	1 : 10	2.500	Field	28/29	840)	159	4	1	2	18	46	54	34	76	4.5
52	1 : 10	2.500	Field	1/18	)	21	15	4	0	0	0	0	2 §	13	0.8
54	1 : 10	?	Field and	79/86	1245)	143	3	4	4	20	33	44	35	74	4.4
54			Laboratory	198/203	)	76	0	0	0	3	8	28	37	88	5.3
55	1 : 10	6.800	Field	121/125	1550	108	0	2	2	10	28	32	34	78	4.7
56	1 : 10	540	Field	134/138	1868	80	2	0	0	2	5	22	49	89	5.4
57	1 : 10	?	Field	49/88	1204	23	11	2	0	0	1	4	5	41	2.4
58	1 : 1	230	Laboratory	207/240	126	68	1	0	0	6	12	20	29	83	5.0
59	1 : 10	?	School & Field	68/84	1680	55	4	0	0	2	6	17	26	82	4.9
60	1 : 10	1.700	Field	100/107	1943	78	1	1	1	7	13	31	24	79	4.8
61	1 : 10	760	School & Field	196/207	3389	99	3	3	1	12	16	32	32	76	4.6
62	1 : 10	1.700	Army & Field	205/218	2866	129	6	1	1	5	14	36	66	83	5.0
63	1 : 10	?	City & Field	134/144	2284	51	1	0	2	2	6	21	19	81	5.0
64	1 : 10	540	Field	78/78	1866	56	3	1	0	3	11	16	22	78	4.8
65	1 : 10	340	City & Field	81/84	1743	73	1	1	2	4	10	29	26	81	4.9
67	1 : 1	540	Laboratory	175/188	73	24	0	0	0	0	7	7	10	85	5.1
68	1 : 10	170	Field	45/71	1293	18	4	0	0	1	5	3	5	64	3.8
69	1 : 5	1.500	Field	57/70	714	10	1	0	0	0	1	1	7	88	5.1
71	1 : 10	170	Army & Field	50/105	2152	47	6	4	0	1	4	9	23	73	4.4
76	1 : 20	850	Field	184/190	4414	93	6	1	1	1	21	24	39	79	4.8
77	1 : 20	850	City & Field	300/317	5933	128	2	1	5	11	22	30	57	81	4.9
78	1 : 10	400	Field	139/149	2807	84	3	0	1	3	10	29	38	85	5.0
79	1 : 5	85	Army & Field	40/51	1486	37	3	0	0	1	3	7	23	85	5.1
80	1 : 5	130	Field	22/37	1785	44	2	0	2	1	3	14	22	83	5.0
83	1 : 10	230	City	270/279	3191	100	2	4	2	5	7	31	49	82	5.0
84	1 : 20	850	Field	37/47	4514	31	0	0	0	0	3	12	16	90	5.4
85	1 : 5	50	City	47/54	2182	43	9	0	0	1	2	11	20	71	4.3
88	1 : 20	850	Field & Lab.	186/202	4752	62	5	1	2	5	6	19	24	82	4.6
95	1 : 20	850	Field	145/163	6233	41	11	3	2	1	2	3	19	59	3.6
101	1 : 10	170	School & Field	212/232	6822	46	0	0	1	0	7	7	31	91	5.5
102	1 : 20	270	City & Field	264/282	7995	70	1	0	1	2	7	11	48	92	5.4
103	1 : 10	100	Field	101/106	3867	40	0	0	0	0	5	11	24	91	5.5
106	1 : 5	85	City	20/23	624	41	13	1	1	1	1	11	13	58	3.5
112	1 : 1	230	Laboratory	85/94	21	14	0	0	0	1	2	5	6	85	5.1
115	1 : 10	110	School & Field	27/42	3614	80	4	1	1	5	5	25	39	83	5.0
117	1 : 10	170	City & Field	150/208	10454	153	18	8	5	9	13	41	59	72	4.2
123	1 : 20	200	Field	118/176	10259	5	0	0	0	0	0	1	4	96	5.8
126	1 : 20	200	City & Field	59/85	6166	120	23	3	6	1	9	30	48	69	4.1
136	1 : 20	220	Laboratory	240/253	9233	9	0	0	0	1	2	3	3	81	4.9
137	1 : 20	280	Field	87/89	7400	38	1	1	0	1	3	17	15	83	5.0
				TOTAL	130897	2944	170	48	48	162	427	858	1231	80	4.8

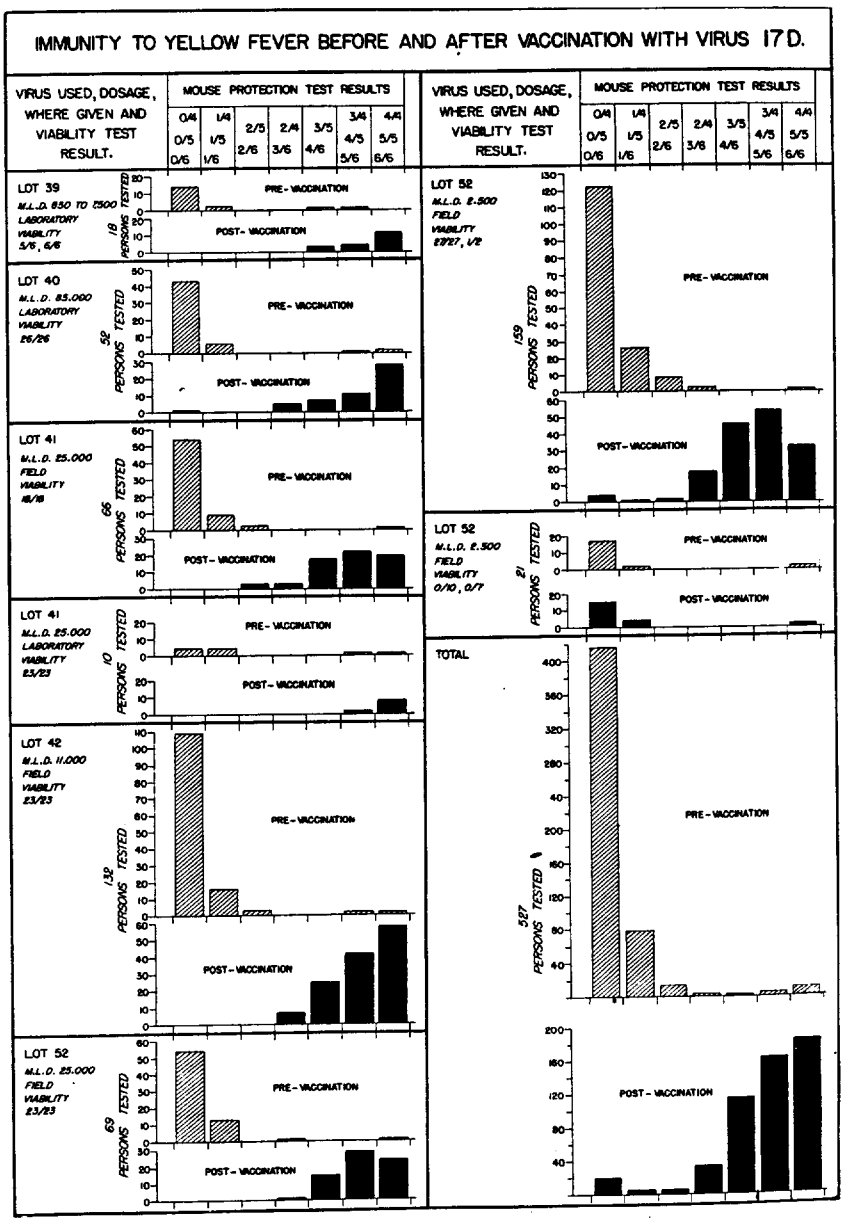
[1] The endpoint for titration of virus in mice is calculated on the basis of 50 % mortality.

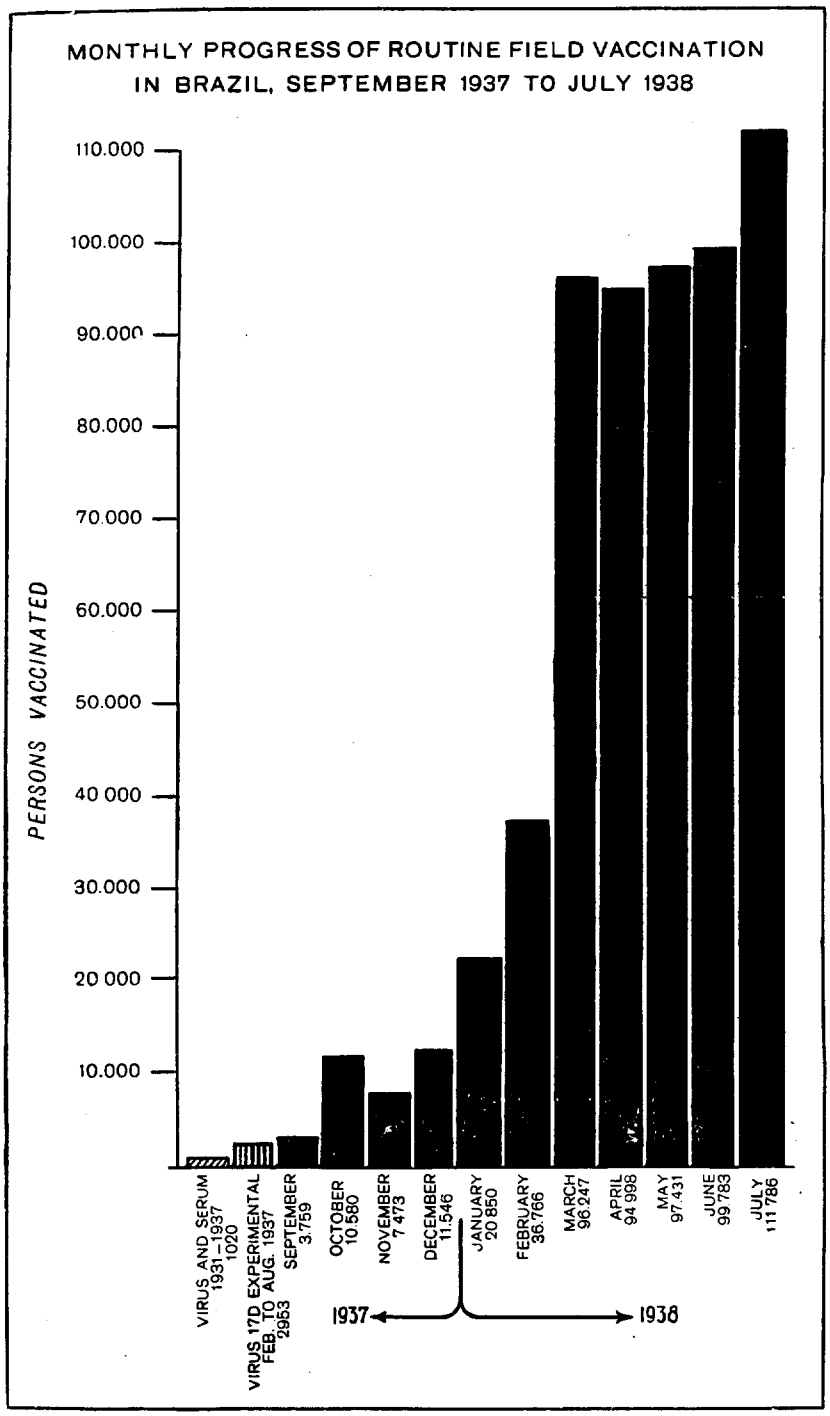
[2] The fraction indicates the number of *mice dying of specific encephalitis* (numerator) in comparison with number alive four days after inoculation (denominator).

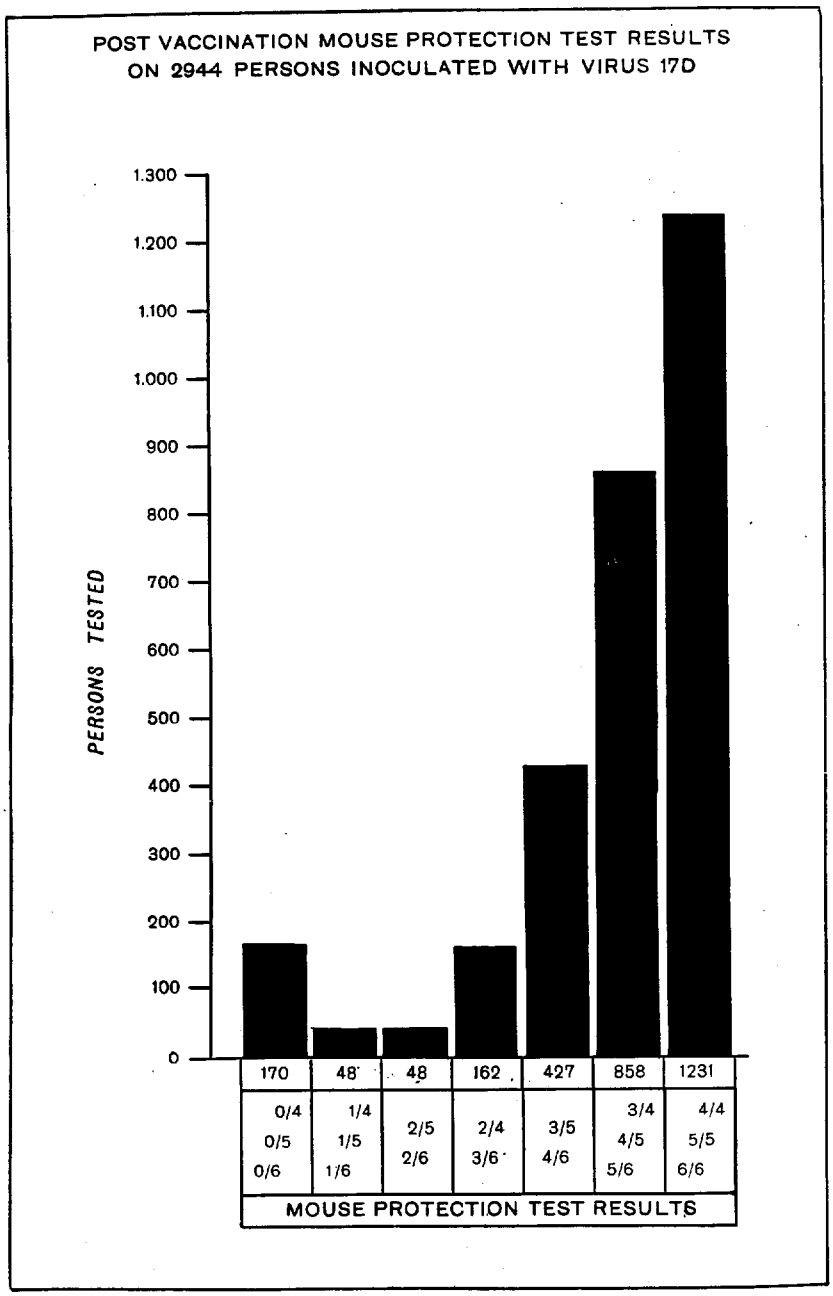
[3] The fraction indicates the number of *mice surviving to the tenth day* (numerator) in comparison with number alive four days after inoculation (denominator).

[4] Average number of mice surviving calculated on basis of six-mouse groups.

§ Prevaccination immunes.


IMMUNITY TO YELLOW FEVER BEFORE AND AFTER VACCINATION WITH VIRUS 17D.
VIRUS USED, DOSAGE, WHERE GIVEN AND VIABILITY TEST RESULT.
MOUSE PROTECTION TEST RESULTS
0/4 0/5 0/6
1/4 1/5 1/6
2/5 2/6
2/4 3/6
3/5 4/6
3/4 4/5 5/6
4/4 5/5 6/6
LOT 39
M.L.D. 650 TO 1500
LABORATORY
VIABILITY
5/6, 6/6
19 PERSONS TESTED
LOT 40
M.L.D. 85,000
LABORATORY
VIABILITY
25/26
52 PERSONS TESTED
LOT 41
M.L.D. 25,000
FIELD
VIABILITY
16/18
66 PERSONS TESTED
LOT 41
M.L.D. 25,000
LABORATORY
VIABILITY
23/23
10 PERSONS TESTED
LOT 42
M.L.D. 11,000
FIELD
VIABILITY
23/23
132 PERSONS TESTED
LOT 52
M.L.D. 25,000
FIELD
VIABILITY
23/23
69 PERSONS TESTED
LOT 52
M.L.D. 2,500
FIELD
VIABILITY
22/27, 1/2
159 PERSONS TESTED
LOT 52
M.L.D. 2,500
FIELD
VIABILITY
0/10, 0/7
21 PERSONS TESTED
TOTAL
587 PERSONS TESTED
PRE-VACCINATION
POST-VACCINATION


MONTHLY PROGRESS OF ROUTINE FIELD VACCINATION
IN BRAZIL, SEPTEMBER 1937 TO JULY 1938
PERSONS VACCINATED
110.000
100.000
90.000
80.000
70.000
60.000
50.000
40 000
30.000
20 000
10.000
VIRUS AND SERUM 1931-1937 1020
VIRUS 17D EXPERIMENTAL FEB. TO AUG. 1937 2953
SEPTEMBER 3.759
OCTOBER 10.580
NOVEMBER 7.473
DECEMBER 11.546
JANUARY 20 850
FEBRUARY 36.766
MARCH 96.247
APRIL 94 998
MAY 97.431
JUNE 99 783
JULY 111 786
1937
1938


POST VACCINATION MOUSE PROTECTION TEST RESULTS
ON 2944 PERSONS INOCULATED WITH VIRUS 17D
PERSONS TESTED
1.300
1.200
1.100
1.000
900
800
700
600
500
400
300
200
100
0
170
48
48
162
427
858
1231
0/4
0/5
0/6
1/4
1/5
1/6
2/5
2/6
2/4
3/6
3/5
4/6
3/4
4/5
5/6
4/4
5/5
6/6
MOUSE PROTECTION TEST RESULTS

Disputatio.

W. A. P. Schüffner (Holland): Mit besonderer Genugtuung hörte ich die Vorschläge Sopers, die er bezügl. des Ablesens des mouse-protection-tests machte. Sie stimmen mit den von uns in Amsterdam gegebenen erfreulich überein. Die Ansprüche, die man an den Mäuse-Versuch stellen muss, haben sich mit der Zeit geändert. Ursprünglich von Theiler und von Sawyer ausgearbeitet, wurden die Methoden von französischer und portugiesischer Seite (im Office international d'hygiène) als nicht spezifisch angegriffen. Um diesen Vorwurf zu entkräften, vermehrte Sawyer die Menge des Virus; statt einer 10% Emulsion nahm er eine 20%; damit konnten unspezifische Reaktionen (die übrigens kaum vorkommen) mit noch grösserer Sicherheit ausgeschaltet werden. Aber natürlich gingen damit schwache spezifische Reaktionen verloren. Heute aber, wo an der Spezifität des Mäuse-Versuchs nicht mehr gezweifelt werden kann, verlangt die Erforschung der Epidemiologie des Gelbfiebers auch das Erfassen einer schwachen Immunität. Wir haben daher einmal die schwächere Emulsion (10%, und davon 0,2 cc. mit 0,4 zu prüfendem Serum intraperitoneal gegeben) beibehalten und zweitens, ebenso wie heute nun auch Soper, vorgeschlagen, die Resultate, die jetzt noch als zweifelhaft oder gar als negativ gelten, mit zu berücksichtigen. Ich stimme Soper vollkommen bei, wenn er daran erinnert, dass selbst ein volkommen negativ abgelaufener Mäuseversuch (6/6) noch nicht eine Rest-Immunität ausschliesst. Zu dieser Auffassung wurden Snijders und ich früher bereits bei unsern Dengue-Untersuchungen gedrungen, später hatten wir sie für das Verständnis der Verhältnisse in Suriname, wo der Eingeborene auffallend resistent bei Gelbfieberepidemien war, nötig.