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NON-FATAL INFECTION OF MICE FOLLOWING INTRACEREBRAL INOCULATION OF YELLOW FEVER VIRUS*

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Although the *rhesus* monkey (*Macaca mulatta*) was the first laboratory animal found to be suitable for experimental work with the virus of yellow fever (1, 2), the laboratory mouse has also played an important rôle in yellow fever research. However, efforts to define more exactly the susceptibility of this animal have been few since the initial demonstration in 1930 that mice could be infected (3) and certain supplementary studies (4, 5) reported shortly thereafter. Basically, these early studies demonstrated the following: that adult mice are highly susceptible to virus inoculated by the intracerebral route (3, 5) but can be infected regularly by extraneural routes only after the brain has been traumatized (4); that baby mice, in contrast, are highly susceptible to virus inoculated extraneurally (3); and that mice of several different strains are not equally susceptible (4, 5).

Recently reported studies of the susceptibility of mice to yellow fever virus have been chiefly concerned with the susceptibility of young mice to extraneurally inoculated virus with special reference to the practicability of their use in protection tests (6) and in testing for the presence of small amounts of virus (7). In studies now being terminated in this laboratory the exact relation of age to the susceptibility of mice to several strains of virus, inoculated by extraneural routes, has been worked out (8), and it has been shown in addition that significant differences related to age exist in the susceptibility of mice inoculated by the intracerebral route, the youngest mice being the most susceptible to infection (9).

The present paper reports observations indicating the occurrence of specific but non-fatal infections among mice inoculated intracerebrally with yellow fever virus. A study has been made of the nature and course of such infections, and of the relation of their occurrence to the strain of virus employed and the dose inoculated.

Materials and Methods

Strains of Yellow Fever Virus.—The observations to be reported were made in large part on mice inoculated with virus of the various 17D substrains which have been

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used as routine in Brazil in the preparation of yellow fever vaccine. The evolution of the parent strain (17D) from Asibi by serial passage in tissue culture, and its major characteristics, were first described in 1936 and 1937 (10, 11). The substrains with which the present report deals are designated in the Rio de Janeiro laboratory as 17DD low, 17DD high, $17D_3$, EP, 17D-NY 104, and 17D-NY 310. The detailed derivation and the individual characteristics of these substrains have been described elsewhere (12–15).

Observations have also been made on mice inoculated with the pantropic Asibi strain; with the mouse-fixed derivative of the French strain first developed by Theiler (3); and with a number of recently isolated strains of pantropic virus, most of which were recovered from human cases in early 1940 during an epidemic of jungle yellow fever in the State of Espírito Santo, Brazil.

Mice.—All of the mice used were of the Swiss strain, bred and raised in the colony of this laboratory from stock imported from a New York breeder 2 and 4 years ago. Mice of exactly known but different ages were used, chiefly within the range of from 21 to 55 days.

Virus Titrations.—Quantitative determinations of virus activity were based upon the intracerebral inoculation of serial fourfold or decimal dilutions of the preparation being studied, into groups of mice, the number of which varied with the experiments. As diluent, human or monkey serum, demonstrated by means of careful protection test studies to possess no virus-neutralizing activity, was employed in 10 per cent concentration in physiological saline. In the intracerebral inoculation of the mice the site (midfrontal area) and depth of inoculation (2 mm.) were carefully controlled to insure the greatest possible uniformity and sensitivity of virus detection.

Inoculated mice were kept regularly for 21 days after inoculation during which time the occurrence of signs of specific infection, fatal or otherwise, was noted. In the normal routine, the titer was then calculated by the 50 per cent end-point method (16) on the basis of the number of mice dying during the period of observation.

Prolonged Observation of Mice.—A large number of mice were collected which had manifested signs suggestive of illness following inoculation with 17D virus but which were still alive 22 days later. Immediately as collected, such mice were carefully examined and classified as to their condition according to the following scheme.

Normal.—A small number of mice had apparently recovered completely from their obvious illness by the 22nd post-inoculation day.

Weak.—Mice in this category showed an impaired ability to resist backward traction across a wire grating, although no definite paralysis could be detected.

Slight Paralysis.—Mice so classified had clearly localized but incomplete paralysis of one or more extremities.

Marked Paralysis.—Such mice showed complete, flaccid paralysis of one or more extremities.

Spastic.—Mice considered in this group had developed a spastic paralysis of one or more extremities. Most commonly, both hind legs were affected, and the condition was associated with the loss of sphincter control.

Sick.—A small number of mice showed the signs often seen at the onset of apparent encephalitis, ruffled fur, a hunched position, reduced activity, but failed to develop definite paralysis.

Atypical.—Mice classified as atypical presented such signs as marked hyperreactivity, weakness associated with marked tremors or restlessness, and atypical ruffling of the fur. Although often thought not to represent examples of yellow fever encephalitis, they could not be excluded with certainty.

As the mice were accumulated and classified, they were formed into groups and individually marked with picric acid and gentian violet for future identification. As necessary, these markings were renewed. The mice were examined subsequently two times a week in order to record any changes in their state, and at various intervals were sacrificed for special studies. Occasionally, this prolonged observation was terminated involuntarily by the appearance of mouse typhoid, which necessitated the immediate disposal of the affected groups.

Detection of Serum Antibody.—At desired intervals mice were exsanguinated from the heart to obtain serum for study; from 0.5 to 1.0 ml. of blood was obtained from each animal. In most cases the blood specimens from several mice were pooled before the serum was separated, although specimens adequate for testing were obtained from a number of individuals. All examinations of sera were made by the intraperitoneal protection test technique in which young mice, in the present case 19 days of age, are employed, and which requires no more than 0.4 ml. of serum (6).

Non-Fatal Infections with 17D Virus

In the course of the many titrations necessary to the careful control of the virus content of individual lots of 17D vaccine, it was noted that not a few mice which had developed typical signs of encephalitis were still alive at the close of the usual 21 day observation period. The study of the eventual fate of such mice and of the true nature of their disease constituted the starting point for the present investigations.

Manifest Infections

Material for the initial studies was obtained by collecting over a period of several months all mice employed in routine vaccine titrations which had shown signs of illness at any time and which were still alive on the 22nd day after inoculation. In all, 543 mice were collected. Examined and classified according to their clinical condition following the criteria indicated in *Materials and Methods*, these mice were held under observation for varying intervals until sacrificed for special studies.

Course of the Disease.—As long as they remained under observation the mice were examined twice weekly to record the course of their disease. The scope of this study is indicated in Table I which shows at succeeding intervals post-inoculation the number of mice which were under observation, which died as the result of encephalitis or of mouse typhoid, and which were deliberately sacrificed.

It is evident from this table that death, due either to the progression of the active encephalitic process, or to the permanent impairment of function re-

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sulting from initial attack, played only a small rôle in bringing the period of observation to a close. Such deaths occurred with greatest frequency before the 31st day post-inoculation, and, although not indicated in the table, were largely among mice which first became ill near the close of the routine observation period. Mice which manifested the first signs of encephalitis before the 15th day post-inoculation and were still alive on the 21st day usually could be kept alive for an indefinite period. At the time the study was terminated 50 mice had been observed for at least 100 days, 10 of these not being sacrificed until the 114th day.

The results of the initial and subsequent examinations are briefly summarized in Table II, both to show the initial distribution of the mice among the several

TABLE I

Continued Observation (beyond the 21st Post-Inoculation Day) of Mice Infected with 17D Virus
Number under Observation, Dying, or Sacrificed during Successive Intervals after Inoculation

		Interval post-inoculation (*days*)									
		22–25	26–30	31–35	36–40	41–50	51–60	61–70	71–80	81–100	100–114
No. of mice under observation		543	481	460	433	394	276	110	73	63	50
Deaths due to specific disease	No.	41	10	5	3	4	0	0	1	1	1
	Per cent	7.6	2.8	1.1	0.7	1.0	0	0	1.4	1.6	2.0
Deaths due to mouse typhoid	No.	0	0	2	0	7	15	15	2	0	5
	Per cent	0	0	0.4	0	1.8	5.4	13.6	2.7	0	10.0
No. of mice sacrificed		21	11	20	36	107	151	22	7	12	44

categories already defined, and to illustrate the changes which occurred in the state of the animals during the period of observation, the length of which, it must be remembered, varied greatly. Further progress of the disease occurred in 92 cases (16.9 per cent), including in this total the 66 deaths believed to have been due, directly or indirectly, to the encephalitis. In contrast, obvious improvement, at times to the point of complete recovery, was observed in 155 mice (28.6 per cent). As the table indicates, such improvement was relatively much more frequent in those mice which had experienced the milder manifestations of the disease, such as weakness, or only slight paralysis, the improvement rates in these two categories being 55.7 and 51.2 per cent, respectively.

Histopathological Examination.—Twenty mice were sacrificed at intervals of from 24 to 114 days post-inoculation to provide material for histological examination. The brains of these mice were fixed in formalin, embedded in paraffin, and sectioned. The sections were stained with hematoxylin and eosin.

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At 24 days signs of a still active process were present, including small areas of hemorrhage and degeneration of the nerve cells. At 31 days perivascular cuffing and lymphocytic infiltration of the meninges were still prominent, the cerebral capillaries seemed engorged, and a glial reaction was evident, but degenerating nerve cells were hard to find. At 38 and 50 days, perivascular cuffing and the meningeal infiltration were decreased but present, and foci of glial cells were easily seen. After the 85th day no cuffing of the vessels could be detected, and the sections examined appeared essentially normal. The histological studies, thus, confirmed the impression gained during life that in most cases little progression of the essential disease process occurred beyond the normal 21 day observation period.

TABLE II

Summary of the Changes in the Clinical State of Mice Surviving 17D Virus Encephalitis

Clinical condition on 22nd day post-inoculation	No. of mice	Subsequent clinical course					
		Disease progressed		Condition unchanged		Condition improved	
		No. of mice	Per cent	No. of mice	Per cent	No. of mice	Per cent
Normal	7	1	14.3	6	85.7	0	0
Weak	113	12	10.6	38	33.6	63	55.7
Slight paralysis	86	15	17.4	27	31.4	44	51.2
Marked paralysis	229	41	17.9	148	64.7	40	17.4
Spastic	84	12	14.3	70	83.3	2	2.4
Sick	13	10	76.9	0	0	3	23.1
Atypical	11	1	9.1	7	63.7	3	27.3
Totals	543	92	16.9	296	54.5	155	28.6

Virus Isolation from the Brains.—Since the mice under study had been inoculated with yellow fever virus of the 17D strain, strong presumptive evidence existed as to the etiology of the disease observed. It remained necessary, however, to exclude the participation of some virus endemic in mice, such as that of spontaneous mouse encephalomyelitis (17).

Initial studies were carried out on mice which had shown signs of illness for from 1 to 11 days. Brains taken during the first 2 days from such mice, prepared as a 10 per cent suspension in saline and inoculated in serial decimal dilutions into new mice, were found to contain an infectious agent demonstrable in dilutions of 10^{-5} or 10^{-6}. Brains taken from the 3rd to the 5th day were found to be infectious irregularly and only in low dilutions, while brains taken after the 5th day were not infectious at all. Although the infectious agent was not specifically identified, its behavior was typical for 17D virus. Furthermore, pooled sera obtained from the same mice were found in all cases to contain protective antibodies against yellow fever.

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In subsequent studies, the brains of 35 additional mice, sacrificed on from the 14th to the 28th day of illness, were examined. These mice included 14 classified as weak, 3 with marked but flaccid paralysis, and 18 which were spastically paralyzed. Material from each brain, suspended in 10 per cent concentration in saline was inoculated into 6 mice which were observed for 6 weeks. In no case was an infectious agent demonstrated.

Serological Immunity.—The disease process in mice which were classified as normal or only weak on the 22nd day post-inoculation was considered to be of particularly uncertain etiology, and a special effort was made to secure serum samples which were adequate for examination from individual animals in these groups. Twelve specimens were obtained, all of which gave clearly positive results in the protection test.

TABLE III

Serum Antibody Levels in Surviving, Intracerebrally Infected Mice at Varying Intervals Following Inoculation

Protection test	Titers of serum pools collected during post-inoculation intervals of: (*days*)									
	22–25	26–30	31–35	36–40	41–50	51–60	61–70	71–80	81–100	100–114
Run 1.....	210	210	170	128 128	256 74	158				
Run 2.....	126		38	98	136	81	132		182	47 39

Studies also were made on serum pools obtained from a much larger number of mice and representing all categories of obvious disease. These pools, each containing sera of from 4 to 8 animals, were titrated in two runs of the protection test, using 12 test mice per fourfold dilution of serum. The titers obtained are shown in Table III with relation to the approximate interval post-inoculation in which the sera were collected. It is evident that protective antibodies against yellow fever virus were present in the sera in clearly significant concentrations up to 114 days post-inoculation, when the observations were terminated.

Resistance to Reinfection.—Although the serological studies had indicated that immunity against yellow fever virus was the rule among mice surviving apparent infection with 17D virus, the immune state of individual mice was verified in a limited number of cases only. In order to multiply the number of observations on individual mice, tests of their resistance to reinfection with virulent French neurotropic virus were employed.

Of the 240 mice which were inoculated intracerebrally with challenge doses of from 10^6 to 10^7 M.L.D. of virulent virus, only 22 failed to survive. Twelve of the deaths occurred among mice in which the original diagnosis was most

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open to question, nine being among a group of 49 mice originally classed as weak, and three being among a group of 5 classed as atypical. Although 9 of 112 mice with marked paralysis also died following the challenge inoculation, death in 8 of these cases was of uncertain cause as it was preceded by atypical signs, by no signs at all, or by evidence suggesting mouse typhoid. The remaining non-resistant mouse was one of 37 animals showing only slight paralysis. Of 6 apparently normal mice and of 31 spastic mice, all successfully withstood the challenge.

Since the great majority of mice (over 90 per cent) surviving an apparent infection following inoculation with material containing 17D virus are resistant to subsequent infection with virulent yellow fever virus, it is at least strongly suggested that their resistance was the direct result of their prior infection, and that the etiological agent of the prior infection was yellow fever virus of the 17D strain.

Inapparent Infections

The clinically apparent but non-fatal disease which presumably resulted from infection with 17D virus varied in severity from an illness resulting in marked and permanent impairment of function to one of but briefly transient nature. That still milder and completely inapparent infections also might occur was an obvious possibility.

Preliminary experiments carried out on apparently normal survivors of titrations of 17D virus soon revealed that a small but significant number of mice were resistant to cerebral infection with highly virulent French neurotropic virus. The absolute regularity with which normal mice of the same stock succumb to the doses of French neurotropic virus which were employed (10^2 to 10^4 M.L.D.) precludes the possibility that the resistance observed was due in an appreciable number of cases to natural resistance of individual mice.

Serological studies were also carried out. Apparently normal survivors from titration groups in which at least one mouse had succumbed with specific encephalitis were sacrificed by bleeding, and the sera obtained from each group were pooled. These pools, each of which contained the sera of from 2 to 4 mice, were then examined by means of the protection test. Of twenty-six pools examined, three were found to contain clearly demonstrable protective antibodies against yellow fever. These serological observations testify to the specific immune basis of the occasional resistance to infection with virulent virus offered by apparently normal survivors of 17D virus titrations, and make it evident that with yellow fever virus of this strain, at least, completely inapparent infection of mice may occur.

The Frequency of Non-Fatal Infections with 17D Virus

The foregoing observations have been based upon a relatively large number of individual examples of non-fatal infection of mice with 17D virus. There

remain for consideration the possible relation of the occurrence of non-fatal infections to the amount and the substrain of the 17D virus inoculated, and also the importance of such infections to the quantitative determination of virus activity.

Relation of Non-Fatal Infections to Virus Dose.—To determine the relation of the occurrence of non-fatal infections to the dose of virus inoculated, the results of 141 routine titrations of 17D virus were reviewed. These titrations, using 12 (or rarely, 6) mice per fourfold dilution, were selected so that none contained less than four infective dilutions. Since none of the mice had been subjected to challenge inocula, the tabulations were limited to the enumeration of animals surviving clinically evident infections.

TABLE IV

Occurrence of Non-Fatal Infections in Mice in Relation to Quantity of 17D Virus (Substrains 17DD Low and EP) Inoculated*

M.L.D.‡ of virus inoculated	No. of mice inoculated	No. of fatal infections	Non-fatal infections		
			No.	Mice inoculated	Mice infected
				per cent	*per cent*
0.1– 0.9	2,336	463	35	1.50	7.03
1.0– 3.9	1,585	1,038	41	2.59	3.80
4.0– 15.9	1,525	1,324	58	3.80	4.19
16.0– 63.9	1,276	1,197	41	3.21	3.31
64.0–255.9	1,029	1,012	10	0.97	0.98
256 or more	138	136	1	0.73	0.73
Totals.....	7,889	5,170	186	2.36	3.48

* Tabulation limited to clinically evident infections. (See text.)

‡ Minimum lethal dose as determined in the titrations in which these mice were used.

As summarized in Table IV, this review demonstrated conclusively that non-fatal infections, at least those evident during life, occur more frequently following the inoculation of small doses of virus. With inocula containing less than 1 M.L.D., 7.03 per cent of the infected mice survived; when between 1 and 63.9 M.L.D. were inoculated, from 3 to 4 per cent survived; and finally, of animals infected with 64 M.L.D. or more, less than 1 per cent survived.

Relation of Non-Fatal Infections to Virus Substrain.—The 17D virus of the 141 routine titrations referred to in the previous section represented but two substrains. Much more suitable data for determining the relation of non-fatal infection to 17D substrain were provided by the results of two experiments in each of which were titrated, under strictly comparable conditions, a number of preparations representing different substrains of virus. In the first experiment, four substrains were studied, using 24 mice (35 to 42 days of age) per tenfold dilution of each preparation. In the second, preparations representing

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six different substrains were titrated on a much larger scale, 102 mice (50 to 52 days of age) being inoculated per fourfold dilution. Within each experiment, the range of infective dilutions examined in mice was very nearly the same for the various substrain preparations. In both experiments, all mice which were still alive on the 28th day after inoculation received an intracerebral challenge inoculum of from 10^3 to 10^4 M.L.D. of French neurotropic virus.

The results of these two experiments are summarized for present purposes in Table V in which the six substrains studied have been listed in the order of

TABLE V

Occurrence of Non-Fatal Infections in Mice in Relation to Substrain of 17D Virus

Virus substrain	Experiment	No. of mice inoculated	No. of fatal infections	Non-fatal infections			
				No. apparent*	No. inapparent‡	Total No.	All infections
							per cent
17DD low	1	118	85	4	2	6	6.6
	2	605	325	17	3	20	5.8
$17D_3$	1	118	91	2	2	4	4.2
	2	596	282	8	10	18	6.0
EP	1	119	99	1	2	3	2.9
	2	595	304	10	1	11	3.5
17DD high	2	608	328	7	3	10	3.0
17D-NY 310	2	607	387	2	4	6	1.5
17D-NY 104	1	118	94	0	0	0	0.0
	2	607	400	1	0	1	0.2

* Apparent infections: paralyzed and resistant to reinoculation.

‡ Inapparent infections: no evidence of disease but resistant to reinoculation.

decreasing frequency of non-fatal infections. The results of both experiments are in essential agreement and indicate that the occurrence of non-fatal infections, apparent and inapparent combined, is significantly related to the substrain of virus employed. Of 495 mice infected with virus of substrain 17D-NY 104, all but 1 succumbed; whereas, of 436 mice infected with 17DD low virus, 26 (or 6 per cent) survived.

Table V also gives some indication of the comparative frequency of apparent and inapparent infections. Of the total of 79 non-fatal infections verified by the demonstration of resistance to reinfection, 27 had produced no manifestations during life. It probably is also of significance that an unusually high proportion of these inapparent infections were produced by virus of substrain $17D_3$.

Significance of Non-Fatal Infections to Quantitative Determinations of Virus Activity.—It is evident that, in so far as virus of a particular substrain produces non-fatal infections in mice, quantitative determinations of its activity based only on mice dying are not fully accurate. For this reason, it has become the practice in this laboratory to administer intracerebral doses of from 10^3 to 10^4 M.L.D. of French neurotropic virus to all mice surviving the usual observation period in those titrations in which full accuracy is of special inportance. Mice resisting such challenge doses are presumed to have been infected by the virus contained in the original preparation titrated, and are included with those whose infection resulted in a fatal encephalitis in the calculation of the titer.

The increase in the accuracy of the titers so obtained varies directly, of course, with the proportion of non-fatal infections produced. Obviously, with virus of substrain 17D-NY 104 the application of the reinoculation technique is of no importance. On the other hand, with virus of substrains 17DD low and $17D_3$ its application may result in a 25 per cent increase in the level of infectivity detected.

Observations with Other Strains of Yellow Fever Virus

Observations with strains of yellow fever virus other than 17D were less extensive since work with these has been conducted on a much smaller scale.

In the case of the French neurotropic strain (of from the 500th to 600th passage in mice) and the pantropic Asibi strain (long maintained by serial passage in *rhesus* monkeys), mice showing grossly detectable signs of illness have not been observed to survive. However, a few silent infections apparently do occur, as is indicated by the fact that occasional mice surviving titrations of virus of these two strains resist reinfection, and also by the fact that pools of sera from such mice have been found to contain protective antibodies. Although the available data do not permit an accurate estimate, it is probable that inapparent infections with these two strains are too infrequent to be of quantitative importance.

Freshly isolated strains from Brazilian cases of jungle yellow fever, however, present quite a different picture. As the result of experience with the more than 100 jungle strains that have been isolated up to the present time, workers in Brazil have long recognized that the susceptibility of mice to unmodified virus of jungle origin may not be uniform. Freshly obtained serum from yellow fever patients has often been observed, when inoculated into mice, to give rise to a mild disease from which the animals recover completely if given the opportunity. In such cases, attempts to initiate passages often were delayed in the expectation that the next day or few days would find the suspect mouse more certainly sick, with the result that, when passage was finally attempted, no virus could be recovered.

In Table VI are recorded observations made on mice inoculated with virus

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of seven strains isolated from patients early in 1940 during an outbreak of jungle yellow fever in the State of Espírito Santo, Brazil. The inocula consisted of serum freshly obtained from the actual patients, or material, serum

TABLE VI

Occurrence of Non-Fatal Infections in Mice Inoculated with Virus Freshly Isolated from Cases of Jungle Yellow Fever

Virus strain*	Source of mouse inoculum (0.03 ml.)	No. of mice inoculated	No. of fatal infections	Study of surviving mice			
				Reinoculation‡		Protection test	
				No. resisting	No. dying	No. of sera pooled	PR§ of pool
AC	Human serum	4	2	0	2	—	—
	Monkey "	4	1	3	0	—	—
	" "	6	5	1	0	—	—
	" "	6	2	4	0	—	—
	Mouse brain	6	5	—	—	1	6/6
JVC	Monkey serum	6	5	0	1	—	—
	Mouse brain	8	7	1	0	—	—
FM	Monkey serum	6	5	1	0	—	—
OC	Human serum	6	3	3	0	—	—
	Monkey "	6	5	1	0	—	—
	Mouse brain	6	1	—	—	5	6/6
AF	Monkey serum	6	5	1	0	—	—
MOF	Monkey serum	6	4	2	0	2	2/6
	Mouse brain	6	2	—	—	2	0/6
MP	Monkey serum	6	4	—	—	2	0/6
	" "	6	4	—	—	2	6/6
	" "	6	2	—	—	4	6/6

* Initials refer to patients from whom virus was isolated.

‡ Reinoculation with 100 M.L.D. of French neurotropic virus, a dose which killed 30 of 31 control mice.

§ PR: protection ratio or number of mice protected *versus* number employed in test.

or brain, from monkeys and mice of the first or second virus passages. Mice surviving the usual period of observation, many of which had shown transient signs of illness, were tested either by cerebral inoculation with French neurotropic virus or by examination of their pooled sera in the protection test. Of 20 mice given a challenge inoculation, 17 resisted reinfection; while of seven serum pools examined, each representing from 1 to 4 mice, four showed clearly

demonstrable protective power, one yielded inconclusive results (probably weakly positive), and two were entirely negative. It thus seems clear that a high proportion of mice inoculated with virus of these jungle strains may develop a non-fatal but immunizing infection.

This frequent survival of infected mice led to considerable difficulty in titrations which were necessary in the course of studies on animal susceptibility and insect transmission with virus of these jungle strains. This difficulty was

TABLE VII

Titrations of Jungle Yellow Fever Virus in Mice
Importance of Considering Non-Fatal Infections

Virus				No. of mice			Titers as based on	
				Infected				
Stra	Preparation	Dilution	Inoculated	Fatally	Total‡	Not infected	Deaths only	Deaths plus non-fatal infections
JZ	Monkey serum	10^{-1}	12	4	11	1		
		10^{-2}	12	4	8	4	60.3	2340
		10^{-3}	12	5	10	2		
		10^{-4}	12	4	4	8		
JZ	Monkey serum	10^{-1}	12	4	12	0		
		10^{-2}	12	8	11	1	31.6	288
		10^{-3}	11	0	0	11		
		10^{-4}	12	0	0	12		
OC	Triturated mouse brain	10^{-1}	11	8	11	0		
		10^{-2}	10	8	10	0	28.2	1410
		10^{-3}	12	5	6	6		
		10^{-4}	12	2	2	10		

* JZ: isolated in Matto Grosso in 1937.
OC: isolated in Espírito Santo in 1940.

‡ Includes non-fatal infections revealed by resistance to an intracerebral challenge with French neurotropic virus.

largely overcome by the application of the reinoculation technique, as is indicated in Table VII in which are presented the results of three typical titrations. The results, in terms of fatal infections only, failed completely to indicate sharp end-points. However, when corrected to include as well the non-fatal infections revealed by reinoculating the surviving mice with neurotropic virus, the results indicated not only adequately sharp end-points but also infective titers of from 9 to nearly 40 times those based on fatal infections alone. These examples well illustrate that the reinoculation technique is absolutely necessary to quantitative work with virus of these jungle strains.

DISCUSSION AND SUMMARY

Observations have been reported which indicate that mice inoculated intracerebrally with active yellow fever virus may develop an infection which is not only non-fatal but may also be completely inapparent.

The most extensive observations were made on mice which showed signs of infection but were still alive 22 days after inoculation with virus of one or another of several 17D substrains. In such cases, the infection usually progressed no further and partial or complete recovery often ensued. Agents other than yellow fever virus were excluded as a significant cause of such non-fatal infections by the failure of repeated attempts to isolate other infective agents, by the demonstration of antibodies against yellow fever virus in the sera of the mice, and by the demonstration of a high degree of resistance on the part of such surviving mice to reinoculation with large doses of neurotropic yellow fever virus.

Completely inapparent infections with 17D virus were also shown to occur. Studies of apparently normal survivors of 17D virus titrations revealed a small but significant number of animals resistant to intracerebral challenge with neurotropic yellow fever virus. Further, pooled sera from such mice were shown to contain specific protective antibodies.

The occurrence of non-fatal infections with 17D virus was found related to virus dose and substrain. Small doses of virus provoked a significantly higher proportion of non-fatal infections than large doses; while different 17D substrains, tested over equivalent ranges of virus dose, varied greatly with respect to the proportion of infections which did not terminate with death. In the case of two substrains (17DD low and $17D_3$), non-fatal infections (as demonstrated by resistance to intracerebral challenge with neurotropic virus) were sufficiently frequent to cause an increase, when included in the computation of the infective titers, of 25 per cent above the figures based on deaths alone. The demonstration of non-fatal infections, thus, may be important to the accuracy of quantitative determinations of infectivity.

Limited observations with virus of the French neurotropic and the pantropic Asibi strains revealed that non-fatal infections do occur, but only rarely.

Somewhat more extensive observations with unmodified virus of strains isolated from Brazilian cases of jungle yellow fever, in contrast, revealed an occurrence of non-fatal infections much greater than that observed with the most productive 17D substrains. With these jungle strains, the demonstration of non-fatal infections proved indispensable to any measure of the level of infectivity of virus preparations.

The demonstration of the proportional occurrence in mice of non-fatal infections with yellow fever virus provides an additional means by which different virus strains and substrains may be characterized.

BIBLIOGRAPHY

1. Stokes, A., Bauer, J. H., and Hudson, N. P., *J. Am. Med. Assn.*, 1928, **90,** 253.
2. Stokes, A., Bauer, J. H., and Hudson, N. P., *Am. J. Trop. Med.*, 1928, **8,** 103.
3. Theiler, M., *Ann. Trop. Med. and Parasitol.*, 1930, **24,** 249.
4. Sawyer, W. A., and Lloyd, W., *J. Exp. Med.*, 1931, **54,** 533.
5. Theiler, M., *Ann. Trop. Med. and Parasitol.*, 1933, **27,** 57.
6. Whitman, L., *Am. J. Trop. Med.*, 1943, **23,** 17.
7. Bugher, J. C., *Am. J. Trop. Med.*, 1941, **21,** 299.
8. Waddell, M. B., unpublished data.
9. Fox, J. P., unpublished data.
10. Lloyd, W., Theiler, M., and Ricci, N. I., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1936, **29,** 481.
11. Theiler, M., and Smith, H. H., *J. Exp. Med.*, 1937, **65,** 767.
12. Fox, J. P., and Penna, H. A., *Am. J. Hyg.*, in press.
13. Soper, F. L., Smith, H. H., and Penna, H. A., *Proc. 3rd Internat. Cong. Microbiol.*, New York, 1939, 351.
14. Fox, J. P., Lennette, E. H., Manso, C., and Souza Aguiar, J. R., *Am. J. Hyg.*, 1942, **36,** 117.
15. Fox, J. P., and Cabral, A. S., *Am. J. Hyg.*, 1943, **37,** 93.
16. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27,** 493.
17. Theiler, M., *J. Exp. Med.*, 1937, **65,** 705.