THE USE OF YELLOW FEVER VIRUS MODIFIED BY IN VITRO CULTIVATION FOR HUMAN IMMUNIZATION

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One of the most striking phenomena to the student of virus diseases is the occurrence of variants. This phenomenon is of particular importance in that several such variants are being used for the immunization of man. The two classical examples of the use of attenuated forms of virus for human vaccination are vaccinia and the fixed virus of rabies. The origin of vaccinia virus is a moot point, but it is almost universally considered to be a variant of smallpox obtained by passage through the cow. This virus was found as such in nature. The fixed virus of rabies was produced by serial propagation in rabbit brain. By this procedure a variant was produced which had lost to a considerable extent its pathogenicity for man and dog.

By means of a comparable method the virus of yellow fever has been modified (1). By serial propagation in mouse brains its viscerotropic affinity is diminished. A highly virulent strain can by this means be readily converted into a relatively avirulent one which nevertheless acts as an efficient immunizing agent. This fact has been made use of in developing methods for immunizing man (2, 3).

However, by serial propagation in mouse brains, the inherent neurotropic affinity of yellow fever virus is markedly enhanced, not only for the mouse, but also for the monkey (4, 5), and probably for man. This increased neurotropism has rendered the virus potentially dangerous for human vaccination. Consequently a search was made to find methods for modifying yellow fever virus—methods which would not only reduce its viscerotropism but would also diminish, or at least not augment, its neurotropism. That this would be possible appeared from the evidence brought forward by the work of Lloyd, Theiler, and Ricci (6), which indicated that by prolonged cultivation in a tissue

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culture medium the viscerotropic affinity of a highly virulent strain of yellow fever virus was markedly diminished, without at the same time producing a marked change in the neurotropic affinity. The virus cultivated in tissue culture was significantly less neurotropic than the virus then in use for human vaccination. This culture virus was consequently substituted (6) for the French neurotropic strain in the method of vaccination used by Sawyer, Kitchen, and Lloyd (2). The attenuation of the viscerotropic affinity produced *in vitro*, however, was not deemed sufficient to warrant the use of this virus without additional protection by the immune serum.

In the previous paper (7) we have presented evidence to show that by the prolonged cultivation in a medium containing minimal amounts of nervous tissue, both of the major affinities are greatly diminished. The virus obtained by propagation in this medium produced extremely mild reactions when inoculated subcutaneously into *rhesus* monkeys. Furthermore, the virus had lost the power of producing fatal encephalitis when injected intracerebrally into these animals. The ability to produce fatal encephalitis in monkeys was lost between the 89th and the 114th subcultures *in vitro*. A comparison of the neurotropic affinities for mice, using the virus obtained from the 114th and the 176th subcultures, showed that the virus from the latter subculture was considerably less neurotropic than that from the earlier. This attenuated virus acted in monkeys as an efficient immunizing agent to a subsequent subcutaneous inoculation of a highly virulent strain. The idea of using this virus without the simultaneous injection of immune serum was obvious. However, before introducing this virus for human vaccination additional experiments were undertaken.

Methods and Materials

The virus used in this study was the so called virus 17 D, which for a long period had been cultivated *in vitro* in a medium containing the tissues of chick embryos from which the head and spinal cord had been removed (7). The methods and materials used in cultivating the virus of yellow fever have been described in previous papers (6, 7). The virus is present in the tissue cultures in rather low concentration. In studying the immune response of monkeys to large amounts of virus, the requisite concentration of virus for inoculation purposes was obtained by utilizing brains of mice which had been infected with tissue culture material.

Vaccines for human use were prepared from infected whole chick embryos inoculated with culture virus by the technique of Elmendorf and Smith (8). This insures a high concentration of virus. Infected chick embryos were ground up in a mortar with normal human serum to make a 10 to 15 per cent suspension. After centrifugation this suspension was passed through a Seitz filter and the filtrate frozen and desiccated in tubes, each containing from 0.5 to 1.0 cc. Aerobic and anaerobic cultures were made on the filtrate to test for bacterial sterility. The potency of the vaccine was tested as routine by titration in mice by intracerebral inoculation.

EXPERIMENTAL

Response of Rhesus Monkeys to a Subcutaneous Inoculation of Various Amounts of Virus.—Two experiments were performed to study the response of *rhesus* monkeys to various amounts of virus.

In the first of these experiments eight monkeys were used. The virus was titrated in mice, and in Table I the estimated number of average lethal doses for mice which each monkey received are shown. The first two monkeys (Nos. 1 and 2) were inoculated with a suspension of infective mouse brains prepared from mice inoculated with material from the 216th subculture. The remaining six monkeys were inoculated with decimal dilutions of the supernatant fluid from the 217th subculture. In the blood of five no virus was demonstrated after inoculation. In three monkeys (Nos. 1, 2, and 4) the presence of virus in the blood was shown for a duration of 1, 2, and 3 days respectively. Two animals (Nos. 1 and 8) responded with a febrile reaction of 1 day's duration, in one animal 1 day after inoculation, and in the other 5 days. All eight animals lived, and seven were shown to have developed neutralizing antibodies in their sera 1 month after inoculation. There seemed to be no correlation between the titer of the serum antibodies and the size of the immunizing dose. One animal (No. 6) failed to become immunized for reasons not apparent, and when inoculated later with a somewhat larger dose of the same tissue culture virus it readily responded with the production of antibodies. The seven monkeys which showed a demonstrable antibody production were given a test dose of French neurotropic virus administered intracerebrally. This method of testing the immunity of a monkey is far more severe than that of inoculating an animal intraperitoneally with the highly virulent Asibi virus, which is generally used. Four of the animals (Nos. 2, 3, 7, and 8) responded to this immunity test with a febrile reaction; two of these developed signs of encephalitis (Nos. 3 and 8), one of which (No. 8) died on the 7th day of yellow fever virus encephalitis. Three normal control monkeys inoculated intracerebrally with the same virus preparation died of encephalitis in 7 to 9 days. It may be significant that the two animals which showed signs of encephalitis following the test dose of neurotropic virus were those which also showed the least amount of neutralizing antibodies.

TABLE I

The Response of Rhesus Monkeys to a Subcutaneous Inoculation of Varying Amounts of Virus 17 D

Monkey No.	Inoculum		Route	Test for virus in circulating blood: mortality ratio in mice inoculated with serum*										Fever on days after inoculation	Results	Antibody titer 30 days after inoculation	Immunity test: French neurotropic virus intracerebrally	
	Sub-culture	Estimated No. of mouse M.L.D.		Days after inoculation													Fever	Results
				1	2	3	4	5	6	7	8	9	10					
																	days	
1	216	73,000,000	i.p.	3/7	0/5	0/4	0/6	0/6	0/6	0/6	0/6	0/4	0/6	5	Lived	1:19	—	Lived
2	"	30,000,000	i.v.	6/6	6/6	0/6	0/5	0/6	0/6	0/6	0/6	0/5	0/6	—	"	1:32	5, 6, 7, 8	"
3	217	13,000	s.c.	0/5	0/7	0/7	0/6	0/5	0/6	0/6	0/6	0/6	0/6	—	"	1:2	6, 7	Encephalitis, lived
4	"	1,300	"	0/5	0/6	0/6	0/6	0/6	2/5	5/6	0/6	0/6	0/6	—	"	1:134	—	Lived
5	"	130	"	0/7	0/6	0/6	0/5	0/6	0/6	0/5	0/6	0/6	0/6	—	"	1:5	—	"
6	"	13	"	0/6	0/5	0/7	0/6	0/6	0/6	0/6	0/6	0/6	0/6	—	"	Negative	Not tested	
7	"	1.3	"	0/5	0/6	0/6	0/6	0/6	0/6	0/5	0/6	0/6	0/6	—	"	1:37	5, 6, 7, 8	Lived
8	"	0.13	"	0/7	0/6	0/4	0/6	0/6	0/6	0/6	0/6	0/6	0/5	1	"	1:2	4, 5, 6	Died of encephalitis

i.p., intraperitoneally.

i.v., intravenously.

s.c., subcutaneously.

* The numerator represents the number of mice that succumbed to infection; the denominator, the number of mice used in the test.

The influence of the quantity of virus in the immunizing dose on the resulting immunity was tested in a second experiment essentially the same as the above.

Six monkeys were inoculated subcutaneously with 1.0 cc. of decimal dilutions of the supernatant fluid of the 215th subculture of the virus in chick embryo tissue. Titration of the virus by intracerebral inoculation of mice showed that the monkeys were inoculated with 0.2 to 20,000 minimum lethal doses for mice. Two animals (Nos. 14 and 16) had a febrile reaction, one (No. 14) of 1 day's duration on the 4th day, and the other (No. 16) of 3 days' duration commencing on the 5th day after inoculation. All the animals lived. The antibody titer

TABLE II

Antibody Response of Rhesus Monkeys Inoculated Subcutaneously with Varying Amounts of Virus

Monkey No.	Inoculum			Serum antibody titer				
	Sub-culture	Route of inoculation	No. of mouse M.L.D.	Before inoculation	Weeks after inoculation			
					4	6	8	12
9	227	s.c.	33,000	0	1:6	1:27	1:33	
10	"	"	33,000	0	1:5	1:33	1:47	
11	215	"	20,000	0	1:8	—*	—	1:4
12	"	"	2,000	0	1:4	—	—	1:3
13	"	"	200	0	1:2	—	—	1:240
14	"	"	20	0	1:2	—	—	1:28
15	"	"	2.0	0	1:2	—	—	1:4
16	"	"	0.2	0	1:8	—	—	1:26

* Not tested.

of sera obtained 1 and 3 months after immunization was determined. The results showed that the antibody titer of the sera obtained 3 months after vaccination was appreciably higher than that obtained after 1 month in four of the six animals. These results are shown in Table II. Included in this table are results of titrations on the sera of two additional monkeys (Nos. 9 and 10) obtained 4, 6, and 8 weeks after vaccination. These two monkeys had been inoculated subcutaneously with 1.0 cc. of a tenfold dilution of a virus preparation representing the 227th subculture. Some of this preparation was later used for human inoculation. As routine in immunizing animals, we have taken the serum antibody titer 1 month after vaccination as an index of the efficacy of the method of immunization. From the results on these animals it is obvious that an entirely erroneous conclusion may be obtained by using this arbitrary time interval, and

it is possible that had the antibody response of all our animals been determined over a longer period of time following inoculation, higher titers would have been found than those recorded throughout this and the previous paper (7).

From the results of these experiments undertaken to determine what influence the quantity of virus in the immunizing inoculation has on the resulting immunity, the conclusion seems warranted that even minimal quantities have the ability to produce an infection and consequently the production of antibodies and immunity. Both monkeys (No. 8, Table I, and No. 16, Table II), inoculated with only fractions of an average lethal dose for mice, developed antibodies. This result seems to suggest that the monkey is probably more susceptible to the virus than the mouse, or at least more uniformly susceptible. Some mice, of the so called Swiss strains used in our work, will become infected even when inoculated with fractions of an average lethal dose. This result is to be expected from the method of determining the average lethal dose, which by definition is the amount of virus which kills half the mice inoculated within a specified period of time.

Time of Appearance of Immunity in Monkeys Following Inoculation. —The time after inoculation when immunity is developed can be determined by two methods. The first is the determination of the time after inoculation when demonstrable antibodies appear in the blood. Antibodies were shown to be present as early as 7 days after inoculation in several monkeys investigated. After 14 days all animals studied had demonstrable antibodies. The second method of determining the time of development of immunity is to inoculate monkeys with a virulent virus at various intervals after the immunizing inoculation. Table III shows the result of such an experiment.

The virus preparation used for the immunity test was a frozen and desiccated serum obtained from a monkey at the height of infection with the virulent Asibi strain. This preparation had been found to be virulent when tested on numerous occasions. All the monkeys used in this experiment were first inoculated subcutaneously with 1 cc. of a 1 in 10 dilution of the tissue culture vaccine. The first monkey in the series (No. 17) received the test dose of Asibi virus immediately after the vaccine. In the other monkeys the interval between the vaccine and the test dose was 1, 3, 5, 7, and 14 days. Two monkeys (Nos. 25 and 26) were inoculated with the test virus alone to serve as controls. Following the inocula-

tion of the Asibi virus, the monkeys were bled daily and the sera obtained injected intracerebrally into mice to determine the presence of virus in the circulation. Monkey 17, which was inoculated simultaneously with the vaccine and the Asibi virus, died of typical yellow fever 3 days after inoculation. Two monkeys (Nos. 18 and 19) which received the test virus 1 and 3 days after the vaccine died of yellow fever 4 and 6 days respectively after the test. Monkey 20, in which the interval between the immunizing and the test inoculation was 5 days,

TABLE III

The Response of Rhesus Monkeys to a Test Dose of Virulent Asibi Virus Injected at Various Time Intervals after Vaccination with Tissue Culture Virus 17 D

Monkey No.	Time interval between vaccination and immunity test with Asibi virus	Test for virus in circulating blood: mortality ratio in mice inoculated with monkey serum							Fever on days after inoculation	Results	Presence of antibodies in serum after vaccination with virus 17 D	
		Days after injection of Asibi virus									7 days	14 days
		1	2	3	4	5	6	7				
	days											
17	0	3/6	7/7	5/5					—	Died on 3rd day		
18	1	0/7	7/7	6/6					3	Died on 4th day		
19	3	0/6	6/6	5/5	7/7				4	Died on 6th day		
20	5	0/7	3/7	0/6	1/5	0/7	0/6	0/6	4, 6	Lived		
21	7	0/6	0/3	0/7	0/6	0/5	0/6		—	"	Negative	Positive
22	7	0/6	0/6	0/7	0/6	0/7	0/5		—	"	"	"
23	14	0/6	0/4	0/6	0/4	0/6	0/5		22	"	"*	"
24	14	0/6	0/6	0/6	0/2	0/6	0/5		18	"	"	"
25	Unvaccinated control	1/4	8/8	8/8	0/5	0/6	0/6	0/5	3, 4, 5	"		
26	" "	4/6	5/6	8/8	5/6	3/6	0/6	0/6	—	"		

* Result inconclusive as three of six mice survived.

showed a febrile reaction but lived. In this animal minimal amounts of virus were demonstrated in the circulation. Four animals (Nos. 21, 22, 23, and 24), in which the intervals were 7 days and 14 days, all lived and showed no virus in their circulation during the first 6 days following the test inoculation. The fevers of 1 day's duration on the 18th and 22nd days following inoculation registered by two monkeys cannot be considered specific. Neutralization tests with the sera obtained from these four animals 1 and 2 weeks after the immunizing injection showed that at the latter period of time demonstrable antibodies were

present in all four animals. The two control monkeys (Nos. 25 and 26) inoculated with the test virus alone unexpectedly lived. Virus was demonstrated in the blood of both, in one animal (No. 25) for 3 days and in the other (No. 26) for 5 days.

In spite of the failure of the two control animals to die, the experiment is not without value. Using presence or absence of circulating virus as an index of immunity, the conclusion seems warranted that a substantial immunity is present 1 week after the immunizing inoculation and a partial immunity in 5 days.

A Comparison of the Pathogenicity of the French Neurotropic Virus and the 17 D Strain of Culture Virus for Experimental Animals.—The two strains of yellow fever virus which have been used to date for the vaccination of man are the French virus, modified by serial mouse brain passage, and the Asibi virus, modified by cultivation in whole mouse embryo.

Of these two, only the first has been used alone, without the simultaneous injection of immune serum (3, 9–11). The pathogenicity of the French neurotropic virus for experimental animals is known, and a certain amount of information is available concerning its pathogenicity for man. *Rhesus* monkeys inoculated intraspinally or intracerebrally with the French neurotropic virus invariably die of yellow fever virus encephalitis (4, 5). Following extraneural inoculation this virus produces an infection which manifests itself by the production of a febrile reaction in approximately 50 per cent of the animals. In Table IV are summarized the results obtained by inoculating twenty-one monkeys by various extraneural routes with varying amounts of French neurotropic virus. Virus in the circulation, lasting for a period of 2 to 6 days, was invariably present; and approximately 30 per cent of the animals died of encephalitis. By mouse brain passage the viscerotropic affinity of the virus has been considerably diminished, whereas the neurotropic affinity has been augmented. It is this enhancement of the neurotropic affinity which makes this virus potentially dangerous for human vaccination. That this fear is justified has been proven by the occurrence of severe involvement of the central nervous system following its use for human vaccination. These neural accidents have occurred both when the virus was administered alone (10, 12) as well as when administered with immune

TABLE IV

The Pathogenicity for Rhesus Monkeys of the French Neurotropic Virus When Injected by Extraneural Routes

Monkey No.	No. of mouse brain passages	Inoculum, mouse brain	Route of injection	Test for virus in circulating blood: mortality ratio in mice inoculated with monkey serum — Days after inoculation										Fever on days after inoculation	Results
		gm.		1	2	3	4	5	6	7	8	9	10		
27	117	0.005	s.c.											3, 6, 7, 10	Lived
28	"	0.005	"											—	"
29	119	0.000,001	"		0/5	0/6		0/6		2/6		2/6		Continuous 14th to 19th	Died on 24th day of encephalitis
30	"	0.000,001	"		2/6	6/6		6/6		0/6		0/6		6th to 9th	" " 11th " " " "
31	"	0.000,001	"		0/6	0/4		0/6		6/6		5/5	7/7	—	" " 10th " " " "
32	176	0.003	"	3/6		2/6		1/6		0/6		0/5		2, 3, 4, 6	Lived
33	"	0.003	"		4/4		0/6		0/6					6, 7	"
34	200	0.01	"	5/11	12/12	11/12	12/12	11/12		1/12		0/11	0/12	10	Died on 12th day, cause undetermined
35	"	0.01	i.p.	0/12	6/11	11/11	11/12	6/12		0/10		0/11	0/12	—	Lived
36	"	0.01	"	7/12	12/12	11/11	12/12	9/11		1/12		0/10		5, 6, 10	"
37	227	0.8	"	7/7	6/6	6/6		2/4		0/6				2, 3, 4	"
38	"	0.0001	"	0/6	0/6	4/6	5/6	3/3		5/6		0/6		9, 12, 13	Died on 9th day, cause undetermined
39	"	0.5	"	6/6	5/5	6/7	6/6	6/6		0/6		0/6		4, 5, 6	Lived
40	"	0.5	"	5/6	6/6	6/7	3/6	5/6		0/3		0/6		5	Lived
41	"	1.5	"	6/6	6/6	0/6	0/6		0/6	0/6				6	Died on 8th day of encephalitis
42	"	1.5	"	6/6	6/6		0/6		0/6		0/6		0/6	—	" " 18th " " "
43	293	0.01	Scarified	0/6										—	Lived
44	"	0.01	"			6/6		4/6		1/6				—	Died on 12th day of encephalitis
45	"	0.01	"			5/6		5/6		0/7				—	" " 12th " " "
46	"	0.01	s.c.	2/6		6/6		6/6		1/6				5, 6, 7	" " 10th " colitis
47	"	0.01	"	5/5		6/6		3/6		0/6				—	" " 11th " encephalitis

serum (13). Not only does the French neurotropic strain occasionally produce severe neural involvement, but the systemic reaction of man to the virus when administered without immune serum is sufficiently severe in a fair proportion of people to contraindicate its use.

The known pathogenic activities for experimental animals of the French neurotropic virus and virus 17 D are summarized in Table V. In all the points enumerated the French neurotropic virus is more pathogenic than the cultivated strain. It is a noteworthy fact, however, that though the French neurotropic virus is extremely pathogenic

TABLE V

A Comparison of the Pathogenicity of French Neurotropic Virus and Virus 17 D for Experimental Animals

Virus	Mice	*Rhesus* monkeys		Hedgehogs
	Average time of death after intracerebral inoculation	Intracerebral inoculation	Extraneural inoculation	Subcutaneous inoculation
	days			
French neurotropic	4–10	Fatal encephalitis	Fever in approximately 50 per cent of animals. Virus present in circulating blood for a period of 2–6 days. Fatal encephalitis in approximately 30 per cent of animals	Death from encephalitis with liver necrosis
Tissue culture virus 17 D	8–20	Non-fatal encephalitis	Occasional fever. Minimal amounts of virus in the circulation: No deaths	Animals survive

for the nervous systems of all the susceptible experimental animals, the number of times the central nervous system has become involved in man following the use of this virus for vaccination is relatively small. The danger of similar accidents occurring in man following the use of the strain cultivated in a medium poor in nervous tissue should accordingly be negligible. After 114 subcultures this virus had lost its power of producing fatal encephalitis in monkeys. That continued cultivation in chick embryo medium after the 114th subculture leads to a further loss of neurotropism is shown by the intracerebral inoculation of mice with later subcultures. From the results

of these animal experiments it is felt that the virus grown in chick embryo tissue for more than 200 subcultures should be safe for human vaccination.

Inoculation of Immune Persons with Tissue Culture Virus 17 D.— As the immune response in monkeys following vaccination with the virus grown for prolonged periods in chick embryo tissue indicated that this response was comparatively mild, it seemed desirable to test the response in immune persons before using this virus for human vaccination. In Table VI is shown the antibody production in four persons inoculated subcutaneously with a vaccine prepared from the 227th subculture of the virus grown in chick embryo tissue. One of

TABLE VI

Antibody Response in Immune Persons to a Subcutaneous Inoculation of Tissue Culture Virus 17 D

Immune persons inoculated	Inoculum			Serum antibody titer										
	Amount	Subculture	No. of mouse M.L.D.	Before inoculation	Weeks after inoculation									
					1	2	3	4	5	6	7	8	9	10
	cc.													
M. T.	1.0	227	330,000	1:32		1:90		1:125		1:110		1:80		1:96
H. S.	1.0	227	330,000	1:2	1:2	1:4	1:3	1:8		1:5		1:21	1:8	
T. F.	0.8	227	70,000	1:2	1:6	1:100	1:100	1:100						
R. L.	0.5	227	44,000	1:2	1:2	1:25	1:20	1:34						

them had an antibody titer of 1 in 32 before inoculation, as a result of an attack of yellow fever several years before. The three other persons had been vaccinated previously, but the antibodies had almost entirely disappeared from their sera. In all four there was a marked antibody response. Apart from a slight local reaction at the site of inoculation no signs or symptoms were noticed.

The antibody response in immune persons to a subcutaneous inoculation of virus grown for a prolonged time in chick embryo tissue differs markedly from the response produced by the French neurotropic virus and virus grown in mouse embryo tissue. Lloyd, Theiler, and Ricci (6) found that the antibody titer in immune human beings, inoculated with the two strains of virus mentioned above, rose

TABLE VII

Results of Vaccination of Non-Immune Persons with Tissue Culture Virus 17 D

Non-immune persons			Virus		Fever on days after inoculation	Highest temperature recorded	Serum antibody titer							
							Before inoculation	Weeks after inoculation						
Laboratory number	Age	Sex	Sub-culture	No. of mouse M.L.D.				1	2	3	4	6	8	12
	yrs.				*day*	*°C.*								
164	45	M	227	50,000	7th	37.2	Neg.	Neg.	1:2	1:8	1:10	1:10	1:3	1:32
166	42	M	"	50,000	1st, 3rd	37.2	"	"	1:2	1:6	1:10	1:3	1:4	
168	40	F	"	50,000	—	37.3	"				Pos.*			
169	42	M	"	50,000	7th	37.3	"				" *			
171	35	F	229	50,000	5th, 6th	37.3, 37.2	"		Pos.*					
172	12	F	"	3,000,000	—		"		" *					
173	35	F	"	3,000,000	—		"		" *					
175	38	F	"	3,000,000	9th, 10th	37.4, 37.2	"		" *					

* Antibody titer not determined.

rapidly to reach a peak on about the 14th day and then rapidly
diminished, so that the titer 4 weeks after inoculation tended to
approximate its initial level. The antibody response of immune per-
sons to the virus grown in chick embryo tissue is much slower. From
the limited number of observations recorded in this paper, it would
appear that the antibody titer rises gradually, the height of the rise
not being reached until the 4th to the 8th week, when a slow decline
sets in. Even after 10 weeks the antibody titer has not fallen to its
initial level.

Inoculation of Non-Immune Persons with Tissue Culture Virus 17 D.
—To date eight normal persons have been vaccinated with the 17 D
culture virus. The vaccines used were prepared from the 227th and
the 229th subcultures. The relevant observations on these persons
are shown in Table VII. The reactions at the site of inoculation
were minimal. Five persons had a febrile reaction, which in four of
them occurred from the 5th to the 7th days. The highest temperature
recorded was 37.4°C. The febrile reactions were accompanied as a
rule by slight headache and backache, which were not severe enough
to prevent the subject from following his normal occupation.

Investigations on the appearance of antibodies showed that, 2
weeks after vaccination, demonstrable antibodies were present in the
serum of all the six persons studied. The sera from three showed no
protective antibodies 1 week after vaccination. The serum antibody
titer has been studied in only two subjects. In both the antibody
titer was determined at intervals after vaccination. The results tend
to show that the antibody titer produced is very low. No information
is available as to the duration of the immunity produced by vac-
cination.

The results obtained in the small number of persons vaccinated with
the tissue culture virus are sufficiently encouraging to warrant a more
extensive trial of the method.

SUMMARY

The response of *rhesus* monkeys to a subcutaneous inoculation with
varying amounts of virus modified by prolonged cultivation *in vitro*
has been studied. The tissue components of the medium consisted
of chick embryo tissue containing minimal amounts of nervous tissue.

The immunity produced in monkeys, as measured by the antibody titer developed, has no relation to the amount of virus inoculated.

Monkeys inoculated subcutaneously with the tissue culture virus are rendered immune to a subsequent injection of a highly virulent yellow fever virus. This resistance is already present 7 days after vaccination.

The subcutaneous inoculation of the culture virus into immune persons leads to a substantial increase of the serum antibody titer.

The results of vaccinating eight normal persons with culture virus are presented. The reactions were minimal. The highest temperature recorded following vaccination was 37.4°C.

The sera taken from the eight vaccinated persons 2 to 4 weeks after inoculation with the tissue culture virus showed the presence of yellow fever antibodies.

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