Serological Responses of Patas Monkeys (*Erythrocebus patas*) to Vaccination Against Cholera and Yellow Fever (37363)

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Gateff (1), after examining sera from a small number of persons in Africa, stated that cholera vaccination (CV) may lower the yellow fever (YF) antibody titers and *vice versa*. Nonhuman primates respond with antibody formation to most vaccines used in man. Patas monkeys (*Erythrocebus patas*) are especially useful in the study of immunologic responses to antigens prepared from vibrios, as well as from yellow fever virus (2). Therefore, this species was used to see whether or not antagonism between CV and yellow fever vaccine (YFV) occurs in primates other than man.

Patas monkeys. Patas monkeys of both sexes, weighing 4–8 kg and free from signs of disease as determined by clinical, hematological, urine, stool, clinical chemistry and blood culture examination, were used. Blood samples were collected before and periodically after inoculations. The sera were stored at —20° without a preservative.

Vibriocidal antibody titer (VA) determinations. Vibriocidal antibody titer was determined by technique of Watanabe *et al.* (3) using *Vibrio cholerae* Ogawa 41 strain. Only 3+ and 4+ reactions were recorded as positive.

Yellow fever neutralizing antibodies. The plaque-neutralization test of Spector and Tauraso (4, 5) was used with the 17D vaccine strain. The neutralizing titers of the sera were recorded in decimal exponents, $\log_{10}$ (Dex) of the highest dilution (6).

All serologic tests were carried out in duplicate.

Vaccination procedure. Cholera vaccination consisted of the intramuscular injection of 4×10^9 phenol-killed and phenol-preserved *V. cholerae* Ogawa 41 organisms; vaccination against yellow fever was by the im injection of ½ the adult human dose of the 17D vaccine. Groups of 4 patas monkeys each were vaccinated with YFV 8, 6, 4, and 2 weeks before, simultaneously with; and 2, 4, 6, and 8 weeks after the administration of cholera vaccine, respectively. Sera were collected before vaccination, then every second week thereafter for VA and Dex determinations for 12 weeks.

Evaluation. Eight patas monkeys were inoculated with CV; a second group of 8 animals received YFV. Blood was drawn at the same intervals as the animals given both immunizing agents. The results of the VA and Dex determinations in these animals served as controls.

The geometric means of the antibody titers of the monkeys in each group and the standard deviation (SD) were calculated. The difference between the means of the antibody titers in two groups was considered statistically significant approximately at the 95% level when their geometric means ± 2 SD did not overlap. The *t* test was used principally as a control.

Results. Tables I and II indicate that when CV and YFV were administered simultaneously, the VA and Dex values were lower than in the respective control sera. If CV was given 2 weeks before YFV (Table I), VA titers were depressed for 4 weeks. When CV was injected 4–8 weeks before YFV, no statistically significant depressions could be shown in the titers by the calculations employed. The administration of YFV simultaneously with or 2 weeks after CV appeared

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TABLE I. Vibriocidal Titers and Dex Values in Patas Monkeys Immunized First Against Yellow Fever then with Cholera Vaccine.

Yellow fever vaccine given weeks after cholera vaccine	Serum examined weeks after completed immunization									
	2		4		6		8		12	
	VA[a]	Dex[b]	VA	Dex	VA	Dex	VA	Dex	VA	Dex
0[c]	980 ± 110	2.3 ± 0.3	870 ± 102	1.9 ± 0.2	570 ± 101	1.7 ± 0.3	<200	1.6 ± 0.2	<200	<1.3
2	1030 ± 120	2.1 ± 0.3	990 ± 113	2.2 ± 0.3	590 ± 112	2.0 ± 0.3	<200	1.9 ± 0.3	<200	1.7 ± 0.4
4	2020 ± 363	3.5 ± 0.5	1310 ± 148	3.1 ± 0.2	740 ± 99	2.8 ± 0.3	<200	2.3 ± 0.3	<200	1.6 ± 0.2
6	2380 ± 376	4.2 ± 0.4	1680 ± 210	3.9 ± 0.3	860 ± 97	2.8 ± 0.4	310 ± 160	2.4 ± 0.3	<200	1.9 ± 0.3
8	2840 ± 275	4.0 ± 0.3	1530 ± 209	3.7 ± 0.3	850 ± 95	3.0 ± 0.4	330 ± 98	2.3 ± 0.3	<200	1.5 ± 0.3
No yellow fever, only cholera vaccine	1940 ± 180	<1.3	1630 ± 255	<1.3	840 ± 93	<1.3	370 ± 98	<1.3	<200	<1.3
No cholera, only yellow fever vaccine	<200	4.1 ± 0.3	<200	3.8 ± 0.3	<200	3.0 ± 0.4	<200	2.7 ± 0.4	<200	1.9 ± 0.5

[a] Geometric means of reciprocal vibriocidal titers to nearest 10 ± standard deviation.
[b] Geometric means of Dex ± standard deviation.
[c] Administered simultaneousy.

TABLE II. Vibriocidal Titers and Dex Values in Patas Monkeys Immunized First Against Cholera then with Yellow Fever Vaccine.[a]

Cholera vaccine given weeks after yellow fever vaccine	Serum examined weeks after completed immunization									
	2		4		6		8		12	
	VA[a]	Dex[b]	VA	Dex	VA	Dex	VA	Dex	VA	Dex
0[c]	870 ± 95	1.9 ± 0.2	730 ± 70	2.1 ± 0.3	430 ± 210	1.5 ± 0.2	<200	1.7 ± 0.2	<200	<1.3
2	880 ± 110	2.3 ± 0.4	710 ± 90	2.0 ± 0.4	400 ± 126	1.4 ± 0.3	<200	1.4 ± 0.3	<200	<1.3
4	1230 ± 205	3.2 ± 0.3	930 ± 98	3.0 ± 0.3	310 ± 87	2.4 ± 0.4	<200	1.9 ± 0.3	<200	1.5 ± 0.2
6	1980 ± 217	3.6 ± 0.6	1210 ± 203	3.3 ± 0.3	780 ± 110	3.1 ± 0.5	380 ± 40	2.4 ± 0.4	<200	1.9 ± 0.2
8	2030 ± 210	2.7 ± 0.4	1470 ± 216	3.5 ± 0.2	890 ± 97	3.3 ± 0.3	350 ± 53	2.8 ± 0.3	<200	2.1 ± 0.2
No yellow fever, only cholera vaccine	1940 ± 180	<1.3	1630 ± 255	<1.3	840 ± 95	<1.3	370 ± 98	<1.3	<200	<1.3
No cholera, only yellow fever vaccine	<200	4.1 ± 0.3	<200	3.8 ± 0.3	<200	3.0 ± 0.4	<200	2.7 ± 0.4	<200	1.9 ± 0.5

[a] Abbreviations as in Table I.

to result in reduced Dex values for 6 to 8 weeks. Decreased IF virus neutralization potency was observed after 6 weeks if both immunizations were carried out simultaneously.

Discussion. Admittedly, the groups of experimental animals were relatively small, and conclusions drawn from laboratory animals, even primates, must be applied cautiously to man. The SD of the mean was sometimes greater than 10%, a value that should not be exceeded in experiments. In some individual animals relatively large variances were reflected as higher Dex titers 8 weeks after vaccination when CV was given simultaneously with YFV. Similar wide spreads of the titers were observed after vaccine administration on the same day in individual animals. Nevertheless differences between the means were always within ± 1 SD in the latter instances when CV and YFV were given at the same time (Tables I and II). Further, the geometric sequence of the serial serum dilutions for the VA test lends itself to considerable differences in the titers from one animal to another. Lower Dex values acquire practical significance when CV is administered after YFV, which is often the case in Africa and Asia. Many disillusionments resulted in cholera research when conclusions were drawn from the results of experiments in rodents, and from disregarding the difference between coproantibodies and circulating antibodies. In this study only circulating VA was determined. Thus the significance of these findings in relationship to cholera in which coproantibodies appear to constitute the first line defense may be debated. However, the Dex values reflect true protective ability against yellow fever which is desirable for the populations of certain extensive areas of Africa and America. Therefore, the lowering of YF neutralizing titers in man after cholera vaccination may be creating a problem. It is possible that not only CV but also other vaccines may influence the Dex titer. Studies of the interaction of CV and YFV in man has been carried out by our group of cooperating laboratories, and the results are being published (7). Individuals receiving YFV and CV simultaneously or less than three weeks apart formed antibodies to a lower titer than those who were inoculated in greater time intervals. There were no synergistic or antagonistic phenomena observed when smallpox and cholera vaccine were given at the same time or within a few weeks. The reason for the bilateral antagonism between CV and YFV is not yet known.

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