mals bearing a unilateral arterial clamp and in the renal cortex of rats overdosed with DOCA on a high sodium diet.

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Trachoma Viruses Isolated in the United States.* 4. Infectivity and Immunogenicity for Monkeys. (26515)

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Monkeys and apes have been the only laboratory animals useful in trachoma research. Baboons and apes have been more susceptible to experimental infection than the lower primates, but even in these animals the experimental disease, produced irregularly by transfer of conjunctival scrapings, has been equivocal—at best a mild follicular conjunctivitis without inclusions. The primate eye has shown more reaction to transfer of material from the closely related disease, inclusion conjunctivitis, developing an experimental disease often acute in onset and more closely resembling the human disease(1).

With egg-grown trachoma virus it became possible to produce regularly in rhesus and cynomolgus monkeys a clear-cut clinical disease with an exudate presenting a characteristic cytologic picture and with inclusion bodies in the conjunctival epithelial cells. It was soon apparent that isolates differed in their pathogenicity for these monkeys, and we have reported experiments on the *qualitative* aspects of this difference as displayed by 2 strains of trachoma virus isolated in the United States(2,3). This report deals with attempts to *quantitate* the infectivity of these strains and to induce resistance to challenge infection.

Materials and methods. Viruses: Three strains of trachoma virus(4) and one strain of inclusion conjunctivitis virus(5) isolated in this laboratory were employed. Several yolk sac passage levels of each strain with the following egg LD_{50} titers (ELD_{50}/ml) were used: Strain BOUR, YS3 to YS12, $10^{4.4}$ to $10^{6.4}$ ELD_{50}/ml; Strain ASGH, YS4 to YS8, $10^{5.8}$ to $10^{7.4}$ ELD_{50}/ml; Strain APACHE #1, YS8, $10^{6.4}$ ELD_{50}/ml; Strain IC-Cal 1 of inclusion conjunctivitis virus, YS9, $10^{5.6}$ ELD_{50}/ml. Stock virus was prepared, stored and titrated as described(6).

Monkey inoculations. For eye challenge, stock virus was diluted in skim milk. Small cotton swabs were soaked in the inoculum and rubbed back and forth 10 times in the upper and lower conjunctival fornices. An

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estimated 0.1 ml of virus inoculum was thus placed onto the conjunctiva. For immunization, monkeys were used which had been infected in the past and had completely recovered, without any residual eye abnormalities. They were injected intramuscularly with 50% suspensions of crude infected yolk sac in broth containing $10^{6.2}$ to $10^{7.4}$ ELD_{50}/ml. Three injections of 0.8, 1.6 and 2.0 ml were given at 6 to 7-day intervals. Eight days after the third injection the monkeys were challenged with a conjunctival inoculum of 0.1 ml BOUR strain estimated to contain $10^{2.4}$ ELD_{50}/ml.

Recording of observations. The animals' eyes were examined at 2 to 4-day intervals with the aid of a focused bright light and a binocular loupe. The examiner washed his hands and flamed instruments after examining each monkey. All examinations were made by 2 of us (C.D. and P.T.) without knowledge of the nature of the inoculum or previous treatment of the monkeys. For each eye examination the following conjunctival changes were recorded on a scale of 0 (absent) to +++ (maximal intensity): hyperemia, discharge, infiltrate and lymphoid follicles. At most clinical examinations, conjunctival scrapings were taken with a platinum spatula, smeared on a glass slide, stained by Giemsa's method and studied for one-half hour each, to establish the cytologic picture and the presence of typical inclusion bodies.

Scoring of results. To quantitate observations and permit comparison of clinical impressions it was necessary to assign numerical values to the recorded data. Of the several conjunctival changes resulting from trachoma virus infection of the monkey eye, lymphoid follicles were the most suitable for quantitation. Their appearance, persistence, number and size could be estimated most accurately by different observers, and their development was highly specific for trachoma-inclusion conjunctivitis virus infection. To minimize inevitable subjective variation in the evaluations by the different observers on different days, the 3 highest follicle scores for both eyes of each monkey during 21 days of inoculation were pooled. By this method, the highest total score obtainable was 18 (3 plus × 2 eyes × 3 observations). Values of Chi square were calculated using a modification for small numbers which gives more conservative values(7).

TABLE I. Dose-Infectivity Relationship of 2 Strains of Trachoma Virus in Cynomolgus Monkeys.

Titer, ELD_{50}/ml	No. monkeys positive/No. inoculated: BOUR strain clinical disease	BOUR strain Inclusion positive smears	ASGH strain clinical disease	ASGH strain Inclusion positive smears
$10^{5.8-6.7}$	3/3	3/3	6/6	5/6
$10^{5.4}$			4/5	1/5
$10^{4.4}$	2/2	2/2	5/5	4/5
$10^{3.4}$	4/4	4/4	2/2	0/2
$10^{2.4}$	4/5	3/5	?/2	0/2
$10^{1.4}$	2/2	1/2		

Results. Infectivity: The largest number of monkey infections were carried out with 2 strains of trachoma virus, BOUR and ASGH. The former was isolated from an acute initial infection in California, the latter from a Pakistani with a relapse of chronic trachoma. The infectivity of these 2 strains for cynomolgus monkey eyes is shown in Table I. On the basis of egg infectivity it can be seen that strain BOUR produces clinical disease more regularly and in higher dilution than strain ASGH. This strain difference is emphasized by the regular and abundant presence of inclusions in BOUR infections and their irregular and sporadic occurrence in ASGH infections. The infectivity of BOUR for cynomolgus eyes appears to be similar to that for embryonated eggs, as $10^{0.4}$ ELD_{50} ($10^{1.4}$ ELD_{50}/ml) produced clinical "monkey trachoma" in 2 of 2 inoculated animals and inclusions in one of them. The data in Table I do not permit estimation of a "minimal infective dose" of either strain for the monkey eye in terms of egg infectivity. However, it is apparent that the average minimal "inclusion producing dose" is about $10^{0.5}$ ELD_{50} of BOUR and more than $10^{3.0}$ ELD_{50} for ASGH.

Severity of infection. Initial observations suggested that strain BOUR was not only able to infect monkey eyes with a smaller inoculum than ASGH but also produced regu-

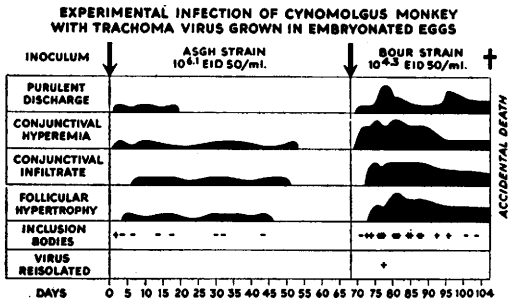

FIG. 1. Different clinical intensity of eye disease resulting from 2 strains of trachoma virus in the same monkey.

larly a more severe and prolonged disease of the eye. This strain difference is illustrated in Fig. 1, which shows the relatively mild disease caused by ASGH followed by a much more intense disease caused by BOUR, in spite of the smaller challenge inoculum employed with the latter strain. To substantiate this impression, follicle scores obtained for all rhesus and cynomolgus monkeys infected with comparable infective doses of these 2 strains are compared in Table IIA. By an arbitrary division of scores it was evident that 10 of 13 BOUR infections were above the half-maximum score, whereas only 2 of 19 ASGH infections fell into that range.

Repeated conjunctival infection. We have described elsewhere(2) that monkeys can be reinfected with the same or a different strain of trachoma virus when signs of the initial infection have subsided. Three such consecutive infections do not appear to modify response to subsequent challenge. In one attempt to superinfect *during* the active phase of experimental trachoma 2 monkeys were inoculated on the conjunctiva with $10^{5.7}$ ELD_{50} BOUR virus within 4 weeks after their inoculation. The animal originally infected with ASGH, however, developed additional disease manifestations with numerous inclusions. The suggestion that some degree of "infection-immunity" might be engendered by the homologous but not the heterologous strain will have to be confirmed in larger numbers of monkeys.

TABLE II. Follicle Scores as Index of Severity of Infection with Trachoma Viruses.

	Score 0-9	Score 10-18	Chi²	P
A. Diff. between strains				
BOUR	3*	10*	12.72	.001
ASGH	17	2		
B. Effect of immunization				
Control	1	3	5.61	.02
Immunized	10	0		

* No. of monkeys in score category; see text for details of experiments.

Effects of parenteral virus administration. In a group of 14 rhesus monkeys we attempted to determine whether repeated large intramuscular doses of live trachoma virus could modify the course of subsequent eye infection. As described above, 10 monkeys received between 10^7 and 10^8 ELD_{50} of live virus of different strains intramuscularly in 3 weekly injections. Four additional monkeys were kept as controls. Eight days after last intramuscular injection all animals were challenged with a conjunctival inoculum of $10^{1.4}$ ELD_{50} strain BOUR ($10^{2.4}$ ELD_{50}/ml). The results are presented in Tables IIB and III.

TABLE III. Effect of Immunization on Rhesus Monkeys to Challenge with $10^{1.4}$ ELD_{50} BOUR Virus ($10^{2.4}$ ELD_{50}/ml).

Immunizing strain	No. infected/No. challenged	Inclusion positive smears	Mean follicle scores
Control	4/4	4	11.5
BOUR	4/4	1	5.0
ASGH	2/2	1	8.25
APACHE #1	2/2	1	8.0
IC Cal 1	2/2	0	5.25

All challenged monkeys manifested some signs of infection, but immunized animals had less clinical activity and few developed inclusion bodies. The follicle scores of immunized monkeys were significantly lower ($p = 0.02$) than those of controls. The details of the experiment (Table III) indicate that only 3 of 10 immunized animals, but all 4 controls, developed demonstrable inclusions, suggesting some limitation of viral multiplication in the immunized animals. The average follicle score for monkeys immunized with any one of 3 strains of trachoma virus (BOUR ASGH, APACHE #1) and one strain of inclusion conjunctivitis virus (IC Cal 1) was lower than for the controls. The most marked reduction in average follicle

score applied to monkeys immunized with BOUR or IC Cal 1. However, numbers are too small to permit interpretation.

Discussion. The results presented here confirm the impression(2) that rhesus and cynomolgus monkeys may serve as useful alternative hosts for study of viruses of the trachoma-inclusion conjunctivitis group. In contrast to the highly variable results obtained with direct "tissue transfer" of conjunctival scrapings from human trachoma to the monkey eye(1), the inoculation of egg-grown viruses resulted in predictable and reproducible disease. There was a general correlation between amount of virus in the inoculum (expressed as ELD_{50}/ml) and rapidity of onset of the disease as well as intensity of clinical signs (hyperemia, discharge, infiltrate and number and size of follicles). However, with increasing dilution of viral inoculum, clinical signs were less well defined. Thus the number of ELD_{50} constituting a minimum "monkey infective dose" could not be accurately estimated on the basis of clinical signs alone.

The more severe the experimental monkey trachoma infection, the greater was the number of inclusion bodies found in conjunctival scrapings. While the microscopic search for inclusion bodies is tedious and time consuming, it lends a measure of objectivity to the other criteria of monkey infection. By such combined assessment it could be unequivocally determined that in terms of ELD_{50} strain BOUR was at least 100 times more infectious for monkeys than strain ASGH, *i.e.,* 100 times more ASGH virus was required to produce a clinical "take" in monkeys with inclusions.

By the "follicle score" method proposed here, differences in severity of infection may be quantitated with some confidence. Why BOUR should regularly produce a more severe disease in monkeys than ASGH, however, remains uncertain. Strains isolated in China(8), Saudi Arabia(9), Gambia(10), and Taiwan(11) tend to produce a mild disease akin to that produced with ASGH. The patient from whom the BOUR strain was isolated in California had no known contact with trachomatous persons, but did develop corneal changes and a pannus entirely typical of trachoma. The greater virulence of monkey infection with BOUR virus is a remarkable strain characteristic, since in the past, viruses of inclusion conjunctivitis have caused a more severe disease of monkey eyes than trachoma. The BOUR strain appears to occupy a unique position in the trachoma-inclusion conjunctivitis group which requires further study.

Like trachoma in humans, experimental trachoma in monkeys did not give rise to demonstrable resistance of the eye to reinfection with the same or a different strain of virus. Very large amounts of virus injected repeatedly intramuscularly did alter the clinical response significantly, suggesting that systemic "immunity" may at times play a role. We have not yet attempted to measure "species-specific" antibodies(12) in these monkeys and cannot comment on their possible role. It will be of interest to examine the effect of parenteral virus administration on the characteristic extended clinical disease and the protracted presence of the virus in the human conjunctiva.

Summary. Three strains of trachoma virus and one strain of inclusion conjunctivitis virus isolated in the United States gave reproducible disease upon inoculation into eyes of rhesus or cynomolgus monkeys. Among several clinical criteria follicle scores were most suitable for quantitation of eye disease, and microscopic demonstration of inclusions in conjunctival scrapings served as additional criterion of infection. Significant differences were demonstrated in the infectivity of 2 strains for the monkey eye. Repeated eye infection failed to modify the response to reinfection. Repeated parenteral administration of live virus in large quantities significantly diminished disease from subsequent challenge infection but failed to induce solid immunity.

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