3- to 4-min measurement period (from labeled 71 to measured 63%), or this may represent slightly reduced saturation in the vial as reported elsewhere.10

Rabbit stromal hydration has been reported between 2.95 and 3.5 g H2O/g dry weight.10 Freeman and Fatt3 have previously reported rabbit stromal Dk to be about 30 × 10^{-11} ml O2 cm2/sec ml mmHg. Our mean figures of 3.38 g H2O/g dry weight and 25.6 ml O2 cm2/sec ml mmHg for hydration and Dk, respectively, agree with the earlier literature.

Ehlers11 found primate stromal hydration to be 3.15 g H2O/g dry weight; use of Hedbys and Mishima’s12 graph of Ytteborg and Dohlman’s13 earlier data leads to a prediction of about 3.0 g H2O/g dry weight for in vivo human tissue. Thus, our mean hydration (about 4 g H2O/g dry weight) for human tissue appears slightly high, as do our thickness values. More that half of our human samples, however, had been stored in fluid for several days prior to freezing, allowing for some fluid imbibition. This analysis suggests, therefore, that human Dk should probably be less than 29 × 10^{-11} ml O2 cm2/sec ml mmHg in vivo.

**Key words:** oxygen permeability, cornea

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An Animal Model of Trachoma: IV. The Failure of Local Imunosuppression to Reveal Inapparent Infection

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Repeated inoculation with live *Chlamydia trachomatis* is necessary to develop a model of trachoma in monkeys. However, it is not possible to reisolate chlamydia from the monkey’s eye after the first 1 or 2 months of weekly reinoculation. The effect of subconjunctival steroid injections in monkeys that had received weekly inoculations with live chlamydia is reported. Despite a profound suppression of local inflammation, steroid treatment did not produce a reactivation of identifiable chlamydial infection as determined by repeated chlamydial cultures and cytologic examinations. Invest Ophthalmol Vis Sci 24:647–650, 1983

Previously we have described the development of an animal model of trachoma.1-3 Our experiments in monkeys have shown that a single inoculation with *Chlamydia trachomatis* produces an acute, self-limited follicular conjunctivitis similar to inclusion conjunctivitis of man, and that after an isolated infection, relative immunity develops, which leads to attenuation of disease after subsequent single challenge.1

Repeated reinoculation is necessary for the initiation and maintenance of chronic follicular disease that is characteristic of trachoma and proceeds to scarring.2 It is the frequency of reinfection and not the serotype of *C. trachomatis* that determines the clinical response.1 Although chlamydia cannot be recovered after the first few months of inoculation, live chlamydia, capable of replication, are necessary for the
development of chronic follicular disease, which is not produced with even the frequent administration of killed organism. Specific antibodies appear promptly in both serum and tears in animals receiving either isolated or repeated inoculation. Serum IgM levels are higher in animals receiving repeated inoculation, and they did not protect against disease. Serum and tear IgA titers are always lower than tear IgG, and again neither immunoglobulin appears to be protective.

We were particularly interested in the necessity for repeated reinoculation, or reinfection, to maintain the chronic disease, even though it is not possible to reisolate chlamydia after the first 1 or 2 months of weekly inoculation. Several authors have commented on the ability of local immunosuppression, particularly local corticosteroids, to reactivate or "light up" persistent chlamydial infection. This report presents our studies using local corticosteroid immunosuppression and its failure to reveal inapparent chlamydial infection.

Materials and Methods. We followed the standard examination protocol described previously using young adult, colony-raised cynomolgus monkeys. The clinical response of each eye was graded for a number of individual signs that we combined to give a simplified "follicular index" to characterize the follicular response, and an "inflammatory index" to summarize the nonspecific signs of inflammation. Tears and serum were collected for serologic tests, smears were obtained from the superior tarsal plate for cytologic study, and conjunctival swabs were taken for chlamydial reisolation cultures. Biopsy specimens were taken from the superior fornix, fixed in alcohol formaldehyde, embedded in JB-4 epoxy (Polysciences, Inc., Warrington, PA), then sectioned and stained with either Lee’s methylene-blue basic fuchsin or Giemsa stain. Although both eyes were examined, specimens were taken from the left eye only to eliminate the possibility of artifactitious changes in the right.

Chlamydia trachomatis, Bour strain, an E-serotype, was grown in yolk sac of embryonated hen eggs and diluted in phosphate buffered saline. Each eye was inoculated with 20 μl of a 10^3 100 ELD50 suspension of chlamydia. Subconjunctival dexamethasone, 2 mg, was injected into the inferior fornix of each eye every second day for 2 weeks in the manner indicated.

Results. The effect of local immunosuppression with subconjunctival steroids was studied in two groups of monkeys. The first group contained five monkeys with "active trachoma" that had received weekly ocular inoculations of chlamydia for 13 weeks. The last positive reisolation cultures were at 6 weeks, and conjunctival chlamydial cultures at 8, 11, and 13 weeks were negative in all five animals. The last weekly inoculation was given on the first day of subconjunctival steroid treatment. The second group of four monkeys had "old trachoma", i.e., previous follicular disease from repeated inoculation.
Fig. 2. Biopsy specimen from the superior fornix conjunctiva of the same monkey as shown in Figure 1, after 2 weeks of subconjunctival steroid treatment. There is a dramatic reduction in the inflammatory response, and only a few residual round cells remain. The epithelium has a normal appearance (original magnification, X200).

with Bour strain, with inoculation stopped 21 weeks previously. Their ocular inflammation had been allowed to resolve before they received steroids.

Chlamydial reisolation cultures were taken from both groups of monkeys at the time of the first steroid injection, which was day 0, and at days 3, 7, 10, 14, 22, 28, 35, and 42. Cultures remained negative throughout the course of steroid treatment. No inclusions were found in conjunctival cytology specimens, although the numbers of inflammatory cells were reduced markedly by 2 weeks in the "active trachoma" group. There was no change in specific antichlamydial antibody titers in serum or tears in either group.

During steroid therapy there was a dramatic decrease in the intensity of the follicular response in animals with "active trachoma", and these clinical changes were apparent on histological examination of conjunctival biopsies. Before the start of steroid treatment, these animals had an intense inflammatory infiltrate in the conjunctiva, with large active germinal centers and many polymorphs and lymphocytes infiltrating the epithelium (Fig. 1). By 1 week there was a substantial reduction in the inflammatory cell infiltrate and a reduction in the size and activity of the germinal centers. By 2 weeks, the conjunctiva in all animals had almost returned to a normal appearance with only a few small resolving follicles (Fig. 2). Although both of the histologic sections that are shown (Figs. 1, 2) are from the same animal, they are representative of the entire tissue block and also of the biopsies obtained from the other animals.

In the animals with "old trachoma" there was no change in the clinical ocular disease, which had already resolved before steroid treatment was started. Histologic specimens were not obtained from the "old trachoma" group.

In our previous studies, repeated inoculations had been given at weekly intervals and the response of all animals within a given group has been similar and consistent. We have recently examined the importance of the interval between reinfections in a group of five animals. These animals had been inoculated weekly for 8 weeks, and all had developed chronic follicular disease. Inoculations were then given every second week for 18 weeks. During this period of biweekly inoculation, the disease in two of the five animals had waned significantly, although they promptly redeveloped follicular disease when weekly inoculation was reinstated. The disease in the other three remained almost constant. At 18 weeks the frequency of inoculation was reduced to once every 4 weeks, and two animals then showed a gradual improvement. These studies indicate individual variation of response to reinoculation when given less often than once per week, although little individual variation is seen when weekly inoculation is maintained. Again, in this study all chlamydial reisolation cultures remained negative after the first few weeks of inoculation.
Discussion. In the early 1950s, several authors reported the reappearance of intracytoplasmic inclusions after topical steroid treatment in patients who had clinically inactive trachoma.4-6 These observations were subsequently confirmed by studies in monkeys that were given steroids after they had made a clinical recovery from an isolated chlamydial infection,7,8 although failures were also reported.9 Evidence of chlamydial infection did not reappear with steroid therapy in our present study, and the reason for the failure to “reactivate” the chlamydial infection is unclear. It is possible that the use of steroids in those previous studies did not in fact “reactivate” the chlamydial infection. In the early studies, steroids were usually tried only in eyes that had active clinical disease and “negative” chlamydial cultures. One explanation for our results could be that improved chlamydial isolation methods now used would eliminate possible false-negative cultures prior to steroid use. Two recent reports have also failed to demonstrate a reactivation or “lighting-up” of chlamydial infection with the use of subconjunctival steroids in guinea pigs and systemic cyclophosphamide in marmosets.10,11

As would be expected, subconjunctival steroids reduced the clinical severity of disease and decreased the inflammatory response, with a dramatic reduction in the cellular infiltrate and in the number and size of germinal centers.

Previously we have shown how repeated inoculation is needed to initiate and maintain chronic follicular disease in our experimental model.1 The response is the same in naive and immune animals. When weekly inoculations are stopped, there is a gradual reduction in disease during the next 2 to 3 months.2 We have also shown, using formalin-killed elementary bodies in huge quantities, that the inoculations must be with live organisms, capable of replication, and that there is no response to normal yolk-sac material.2,3 The present study confirmed that despite the establishment of chronic disease with live chlamydia, we were not able to reisolate the agent or to demonstrate its presence in either cytological or histological specimens, even with the provocative use of local corticosteroids.

The failure to demonstrate chlamydia would argue against the likelihood of “persistent” chlamydial infection in our model, and it suggests that brief but repeated exposure to chlamydial antigens may be all that is needed to continue the inflammatory response. If this is so, however, it is hard to explain the lack of response to killed antigen, unless one also postulates that the chlamydial agent must first enter the epithelial cells before it can act as an antigenic stimulant. It has been shown that heat-inactivated elementary bodies are not phagocytosed whereas ultraviolet-irradiated elementary bodies, even though they are unable to replicate, are able to enter cells.12 It seems likely that formalin-inactivation has an effect comparable to heat-inactivation and prevents elementary body phagocytosis.

The apparent paradox of the need for repeated exposure to live organisms rather than killed antigen, as well as the failure to demonstrate persisting infection in this model, requires further investigation.

Key words: chlamydia, trachoma, animal model, persistent infection, corticosteroid, inflammation

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