Ocular infection of rabbits with a Bedsonia isolated from a patient with Reiter’s syndrome

H. Bruce Ostler, Julius Schachter, and Chandler R. Dawson

Eye disease, consisting of a papillary conjunctivitis, corneal edema, corneal opacity, corneal neovascularization, and iritis, was readily produced in New Zealand white rabbits by the intracameral inoculation of a Bedsonia recovered from the synovial membrane of a patient with Reiter’s syndrome. No eye disease could be produced by the instillation of the agent into the rabbit's conjunctival sac. The agent was found to persist in the conjunctiva for at least 23 days and in the anterior chamber for at least 68 days. It spread to the liver, lung, spleen, and joints. Arthritis was noted in one of 6 rabbits inoculated conjunctivally and in 2 of 8 rabbits inoculated with high titers of the agent intracameraly. In one rabbit in each of these groups, behavior indicating central nervous system changes was also noted.

Key words: Reiter’s syndrome, Bedsoniae, pathogenicity, conjunctivitis, iritis, corneal opacity, corneal neovascularization, corneal edema, arthritis, anterior chamber injections, subconjunctival injection, rabbits, chlamydia, uveitis.

Recently Schachter and associates reported the isolation and propagation of a bedsonial (chlamydial) agent from synovial fluid, synovial membranes, urethras, and conjunctivae of patients with Reiter’s syndrome. Intra-articular inoculation of rabbits with the agent produced arthritis as observed by Smith and associates. As a further step in relating the agent to Reiter’s syndrome, which is manifested in the eye by both conjunctivitis and iritis, the present investigation was undertaken to determine the agent’s pathogenicity for the eyes of rabbits.

Materials and methods

Animals. The experimental animals were 34 randomly bred and selected New Zealand white rabbits whose freedom from eye disease prior to inoculation was established by slit lamp examination.

Inoculum. The agent (25-SM) was isolated in 1964 from a synovial membrane of a patient with Reiter’s syndrome. The method of propagating this agent in the yolk sacs of 7-day-
old embryonated hens' eggs was described in previous communications. A single frozen pool of the twenty-third yolk sac passage was used in all experiments. The material was kept frozen until 15 minutes prior to use. Before and after animal inoculation, all aliquots of the agent were tested for bacterial sterility on thioglycolate broth.

**Inoculation procedure.** The eyes were anesthetized by proparacaine hydrochloride 0.5 per cent* and proptosed with a sterile, cotton-tipped applicator. In most experiments, one eye of each rabbit received agent and the other eye received normal yolk sac. To inoculate the conjunctiva, we instilled 0.1 ml. of the agent (or normal yolk sac) into the conjunctival sac. To inoculate the anterior chamber, we injected 0.1 ml. of the agent (or normal yolk sac) through a 27-gauge needle that had pierced the cornea obliquely 2 mm. anterior to the limbus at the 3 or 9 o'clock position.

**Experimental design.** A total of 8 groups of rabbits were inoculated variously as follows:

- **Group 1** was inoculated conjunctivally with $10^4$ egg lethal doses (ELD$_{50}$) of agent in the right eye and with normal yolk sac in the left.
- **Groups 2, 3, and 4** were inoculated intracameraly with agent (in concentrations of $10^4$, $10^2$, and $10^3$ ELD$_{50}$ respectively) in the right eye and with normal yolk sac (diluted for Groups 3 and 4) in the left.
- **Group 5**, composed of 2 animals from Group 2, was reinoculated intracameraly with normal yolk sac in the left eye after all evidence of the earlier inoculation had subsided.
- **Group 6**, composed of 3 animals from Group 3, was reinoculated intracameraly with agent ($10^4$ ELD$_{50}$) in the left eye after all evidence of the earlier inoculation had subsided.
- **Group 7** (a control group) was inoculated intracameraly in the right eye with normal yolk sac.
- **Group 8** (a second control group) was inoculated intracameraly with heat-inactivated agent ($10^4$ ELD$_{50}$) in the right eye and with heat-treated normal yolk sac in the left.

A summary of this experimental plan is presented in Table I.

**Heat inactivation of the agent.** To inactivate the agent, we boiled it for 20 minutes. Inactivation was proven by serial blind passage of the heated agent in embryonated hens' eggs.

**Clinical observations.** Each inoculated animal was examined with the slit lamp by the same investigator each day for the first week, twice a week for the following 2 weeks, and once a week for the remaining 23 weeks. At each exami-
nation 9 specific signs were noted: (1) discharge, (2) conjunctival hyperemia, (3) follicle formation, (4) corneal staining with fluorescein, (5) corneal opacification, (6) neovascularization, (7) aqueous flare, (8) cells in the aqueous, and (9) fibrin in the aqueous.

Each animal was examined periodically for joint changes by Drs. D. E. Smith and P. James, who had studied arthritis in rabbits injected intra-articularly with the same agent. Arthritis was diagnosed on the basis of heat, redness, swelling, and tenderness (shown by resistance to passive movement of the joint in question).

Serology. Complement-fixation tests for the psittacosis-lymphogranuloma venerium-trachoma group of organisms were performed on all rabbits before inoculation with the agent and 63 days after inoculation, according to the method of Meyer and Eddie.

Pathology. Most of the rabbits that died during the period of observation were subjected to autopsy. The kidneys, liver, lungs, spleen, and eyes were examinedgrossly, and portions of each were removed for histologic sectioning, bacterial cultivation, and reisolation attempts. The portions removed for histologic sectioning were fixed in 10 per cent buffered formalin and stained with hematoxylin and eosin and with MacCallum’s stain.

Reisolation attempts. All specimens were identified by code number only. Control specimens were treated in the same way as those inoculated with agent.

The specimens for reisolation attempts were obtained as follows: (1) from the conjunctival sac, by scraping the lower palpebral conjunctiva with a sterile platinum spatula, and (2) from the anterior chamber, by introducing a 20 gauge needle by means of the same technique used in making the inoculation. Each specimen was transferred to 1.0 ml. of broth containing 2.5 mg. of streptomycin, 0.5 mg. of ristocetin, 0.5 mg. of neomycin, and 100 units of nystatin. After being permitted to stand at room temperature for an hour, the broth suspension was inoculated into the yolk sacs of embryonated hens’ eggs.

When the animals died, attempts were made to reisolate the agent from their livers, spleens, kidneys, lungs, and eyes by grinding specimens with carborundum to make a 50 per cent suspension of tissue with antibiotic broth as described in a previous publication. Bacterial cultures of material from the same organs were made by touching the tissue’s cut edge to blood agar plates. The only joints from which attempts were made to reisolate the agent were those showing clinical evidence of arthritis. For each such reisolation attempt, the animal was skinned, the joint was opened, and portions of the synovial membrane, capsule, and cartilage were removed.

<table>
<thead>
<tr>
<th>Locus of recovery attempts</th>
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<tbody>
<tr>
<td>Anterior chamber</td>
<td>1:2</td>
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<tr>
<td>Conjunctiva</td>
<td>2:3</td>
</tr>
<tr>
<td>Joint</td>
<td>0:1</td>
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<tr>
<td>Liver</td>
<td>1:3 1:3†</td>
</tr>
<tr>
<td>Lung</td>
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<tr>
<td>Spleen</td>
<td>0:1</td>
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<tr>
<td>Brain</td>
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<td>Blood</td>
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*Agent was recovered as early as one week and as late as 9 weeks after inoculation.
†In one animal agent could not be isolated from the liver, but was isolated from a mass attached to the liver.

Results

Group 1. Conjunctival inoculation. No detectable eye changes were noted in any of this group of rabbits during the 6 month period of observation. On postinoculation day 49, one animal developed an acute arthritis of the right rear phalangeal joint, which persisted until death 14 days later. One day prior to death (postinoculation day 62), this rabbit apparently developed central nervous system changes. Although previously a docile animal that was easy to handle, it now made aggressive attempts to bite the handler.

Of 3 attempts to reisolate the agent from the conjunctivas of 3 animals in this group, 2 were successful. The agent persisted in both the liver and anterior chamber for as long as 63 days after inoculation. The results of the reisolation attempts are summarized in Table II.

In one animal an encapsulated, noncaseous mass attached to the liver was found at autopsy. Simultaneous reisolation attempts resulted in recovery of the agent from the mass but not from the liver. Bacterial cultures of material from the mass were also positive for S. Aureus.

Group 2. Intracameral inoculation of 10³ ELD₅₀. Of the 8 inoculated right eyes in this group of rabbits, 7 developed inflammatory eye disease during the period of observation. Changes that were first noted 12 hours after inoculation became
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very severe within 48 hours: their onset and duration are presented graphically in Fig. 1. The agent was reisolated from the anterior chamber, liver, joint, and spleen at various intervals up to 6 weeks after inoculation. The results of reisolation attempts are summarized in Table III.

The experimental ocular disease. The sequence of changes in the experimental disease was as follows: a papillary conjunctivitis with mucopurulent discharge developed 24 hours after inoculation and persisted for an average of 19 days. From the first day, iritis manifested by cells, flare, and fibrin in the anterior chamber was also present. A concomitant corneal opacity developed so rapidly that the only way to judge the duration of the iritis was by following the circumcorneal hyperemia. The opacity progressed until it involved the entire cornea within 5 days, and on the average the iritis persisted for 12 days. In 2 eyes, the corneal periphery cleared by postinoculation day 35 but remained opaque, centrally. In the other eyes, the entire cornea remained opaque throughout the period of observation (Fig. 1).

Table III. Recovery of Bedsonia from animals inoculated in the anterior chamber*

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*Agent was recovered as early as one week and as late as 6 weeks after inoculation.

The cornea perforated on postinoculation day 2 in one of the 8 eyes. The perforation occurred at the limbus, away from the site of inoculation, and healed spontaneously. On postinoculation day 5, corneal ulceration developed in one eye; it was superficial, covered less than one eighth of the corneal diameter, and healed after 5 days without additional scarring of the already opaque cornea.

Between postinoculation days 7 and 13 (with day 12 the average), superficial and midstromal neovascularization of the cornea was noted. It advanced until it
reached the center of the cornea (about postinoculation day 28). The vessels then diminished in size and the entire inflammatory process subsided spontaneously.

In one of the 8 animals, a recurrent conjunctivitis, associated with persistent rhinitis, was noted on postinoculation day 119. Bacterial cultures of material from the eye were sterile. Attempts to reisolate the agent from the eyes, liver, and kidneys of this animal 6 months after the original exposure were negative.

Systemic changes. Acute arthritis developed in 2 rabbits. On postinoculation day 30, one animal developed an arthritis of the carpal joints that persisted until the animal was killed 6 days later (Figs. 2 and 3). On day 89, a second animal developed an arthritis of the phalangeal joints that persisted until the animal died on day 93.

In one rabbit, central nervous system changes similar to those noted in the animal in Group 1, appeared on day 92.

Group 3. Intracameral inoculation of $10^4$ ELD$_{50}$. Like the 7 rabbits in Group 2, 4 of the 6 rabbits in Group 3 developed iritis, conjunctivitis, and corneal opacity. Clinically, the conjunctivitis and iritis seemed to be less severe than in Group 2.

Group 4. Intracameral inoculation of $10^1$ ELD$_{50}$. Only one of the 6 rabbits in this group developed the conjunctivitis, corneal opacity, and iritis that characterized the disease in the rabbits in Group 2. The severity of the induced disease seemed to be about the same as in Group 3.

Group 5. Reinoculation of previously infected animals with normal yolk sac. No inflammatory disease was noted in the left eye and no evidence of a recurrence of inflammation was noted in the right eye.

Group 6. Reinoculation of previously
**Fig. 4.** Section of injected eye through angle of anterior chamber 8 days after inoculation. Note accumulation of inflammatory cells at angle. (x80.)

**Infected animals with** $10^4 \text{ELD}_{50}$. No evidence of reactivation of the inflammatory process was noted in the right eye, but typical disease was produced in the left eye when it was reinoculated, this time with agent.

**Group 7. Intracameral inoculation of normal yolk sac.** Agent was not recovered from any of the control animals, and no control animal showed evidence of eye disease or arthritis during the period of observation. No eye disease was noted in any of the eyes receiving normal yolk sac material.

**Group 8. Intracameral inoculation of heat-inactivated agent.** No detectable disease developed in the eyes inoculated with heat-inactivated agent or with heat-treated normal yolk sac during the period of observation.

**Calculation of infective dose (Kärber method).** The concentration of organisms necessary to produce an iritis of 5 or more days' duration in 50 per cent of rabbits injected intracameraly was calculated by the Kärber method and found to be approximately $50 \text{ELD}_{50}$.

**Histological findings.** The inflammatory reaction inside the eye seemed to be localized predominantly to the trabecular area and to the endothelium of the cornea. There was surprisingly little ciliary body reaction (Fig. 4). In addition to the predominant mononuclear cells, there were a moderate number of large cells containing what appeared to be phagocytized material.

**Serological findings.** All of the animals had CF titers of less than 1:2 prior to inoculation with the agent and only insignificant CF titers on postinoculation day 63.

**Discussion**

Reiter's syndrome appears classically as a triad of disease manifestations: urethritis, arthritis, and iritis, or conjunctivitis or both. By intracameral injection of $10^4 \text{ELD}_{50}$ of a Bedsonia agent (25-SM) isolated from the synovial membrane of a patient with Reiter's syndrome, we were able to produce an iritis and conjunctivitis in 7 of 8 rabbits, and an arthritis in 2 of the 8. Corneal edema and opacification were fol-
ollowed by superficial neovascularization of the cornea, and in one instance by superficial corneal ulceration.

Strains of herpes simplex virus introduced into the anterior chamber produce an acute inflammation that begins in one day, clouds the cornea, and subsides within 2 weeks. The severity of this herpetic inflammation is similar to that produced by isolate 25-SM, but its duration is shorter.

Other Bedsoniae have also been inoculated into the anterior chamber, with the following results: (1) a TRIC agent (IC Cal 3) has produced minimal inflammatory changes that have subsided within 4 or 5 days, (2) the T'ang strain of trachoma agent has produced an ocular reaction clinically similar to but milder than the reaction to agent 25-SM described above, and (3) an ornithosis agent has produced a similar inflammatory reaction except that minimal inflammation was noted in the iris.

Nonpathogenic bacteria introduced into the anterior chambers of rabbits produce minimal inflammation. Pathogenic bacteria, on the other hand, produce marked inflammation that begins on the second or third day, and progresses rapidly to rupture of the globe and phthisis.

In a group of animals receiving the bedsonial agent by conjunctival inoculation, no detectable eye disease was noted, but the agent was recovered from the conjunctiva, anterior chamber, liver, and a liver mass, and one rabbit developed monartricular arthritis. This finding indicates that 25-SM is capable of establishing a systemic infection after conjunctival inoculation.

The rapidity and explosive nature of the onset of the eye manifestations suggest that the early changes noted in the anterior chamber may well have been due to some direct toxic effect of the living organism: no inflammation whatever was noted in eyes that received similar dilutions of heat-activated agent or heat-treated normal yolk sac.

There was no demonstrable evidence of a hypersensitivity component: (1) neither heat-inactivated nor live agent introduced into the opposite eye of a rabbit previously exposed to the agent caused signs of inflammation in the previously inflamed eye, and (2) attempts to reactivate the disease in the contralateral eye by injecting agent into the ipsilateral anterior chamber were unsuccessful.

The fact that one rabbit failed to show evidence of inflammation in the anterior chamber but later showed signs of arthritis suggested that individual variation in host response may play a role.

We wish to express our appreciation to Drs. D. E. Smith and P. G. James for examining the animals.

**REFERENCES**


