

Isolation of Bedsoniae from the Joints of Patients with Reiter's Syndrome.* (31113)

J. SCHACHTER, M. G. BARNES, J. P. JONES, JR., E. P. ENGLEMAN AND K. F. MEYER
*George Williams Hooper Foundation, Francis I. Proctor Foundation, and Rheumatic Disease Group
of Department of Medicine, University of California Medical Center, San Francisco*

This study is concerned with the possible role of bedsonia (psittacosis-lymphogranuloma venereum-trachoma group of microorganisms) in human arthritis. A member of the bedsonia group caused polyarthritis in lambs (1). Arthritis has also been reported as a rare complication of lymphogranuloma venereum (LGV). Three members of the bedsonia group, the agents of trachoma, inclusion conjunctivitis and LGV, are known to infect the conjunctiva and/or genitourinary tract in man. Siboulet and Galistin(2) claimed to have isolated a large trachoma-like virus from the urethra of patients with Reiter's disease, a syndrome consisting of arthritis, urethritis and nonfollicular conjunctivitis(3). Since they were unable to maintain the isolate, however, the agent could not be identified with certainty. Subsequently, Jones(4) reported isolation of inclusion conjunctivitis agents from the urethra of male patients with urethritis, suggesting that isolation of bedsoniae from patients with nonspecific urethritis would not be unexpected.

These observations prompted the present investigation of patients with Reiter's syndrome. This report describes the results of preliminary studies designed to gain serologic evidence of infection and to isolate the infective agent from the synovial tissues, urethra and conjunctiva of such patients.

Materials and methods. The subjects were 16 patients with Reiter's syndrome all of whom had arthritis and urethritis and/or conjunctivitis. Specimens for bedsonia isolation attempts were obtained from 8 of the patients. Serum was collected from all patients for complement fixation tests. Fifteen patients with osteoarthritis or rheumatoid arthritis served as a control group.

* This work was supported by USPHS Grants AI-04759, GM-11329 and 2A-5124 and by funds from Research to Prevent Blindness, Inc., and the Arthritis Foundation.

Isolation studies. Urethral and conjunctival scrapings were obtained from 7 patients. The specimens were taken with special platinum spatulas after topical anesthesia and collected in 1 ml of nutrient broth containing 2.5 mg streptomycin, 0.5 mg neomycin, 0.5 mg ristocetin and 100 units nystatin. Synovial specimens were collected by punch or open surgical biopsy from 4 patients and from the 15 control subjects. The membranes were placed in 1 ml of sterile nutrient broth; they were ground with carborundum before inoculation into eggs.

The test specimens were inoculated into the yolk sacs of 7-day-old embryonated hens' eggs, incubated at 37°C before inoculation and at 34°C thereafter. Eggs still viable at 20 days were killed by chilling. The yolk sacs were harvested, ground and prepared as 50% suspensions in nutrient broth. One ml of suspension was used for blind passage. The suspensions were tested for sterility by culturing on thioglycollate broth and blood plates before each passage.

The results were considered positive when regular egg lethality developed, characteristic elementary bodies were seen in yolk-sac smears stained by a modified Macchiavello technic(5), the agent was maintained by serial passage, and the bedsonia group antigen was demonstrated in the infected yolk sacs. For the latter purpose a 20% yolk-sac suspension was steamed for 1 hour, then cooled. Doubling dilutions of the suspension were then tested for complement fixation against 4 units of human convalescent psittacosis antiserum. If no evidence of bedsonia was demonstrable by the end of the second passage, the isolation attempt was terminated.

Cytology. Direct impression smears of the test specimens were stained by the Giemsa method and by a direct fluorescent antibody stain using guinea-pig antiserum against the

TABLE I. Complement Fixation (CF) Titers and Isolation of Bedsoniae in 5 Patients with Reiter's Syndrome.

Patient No.*	CF titer†	Bedsonia isolation			
		Synovial membrane	Synovial fluid	Urethra	Conjunctiva
25‡	1:32	+	—	—	—
65	<1:2	n.d.	n.d.	+	+
74	1:4	+	+	n.d.	n.d.
98	1:128	+	—	+	—
108‡	1:128	n.d.	+	—	—

* Patients are identified by numbers assigned sequentially within a large series of bedsonia isolation studies being performed at the Hooper Foundation.

† Dilution of serum yielding positive complement fixation in presence of psittacosis group antigen; a titer of 1:16 was considered positive.

‡ Some exposure to tetracycline before specimens were collected.

+ = positive; — = negative; n.d. = not done.

cell wall of an inclusion conjunctivitis agent (6).

Serology. Complement fixation (CF) tests were performed on sera from patients and control subjects as described by Meyer and Eddie(5). The antigen was a boiled phenolyzed yolk-sac suspension of the psittacosis isolate 6BC. A serum titer of 1:16 was considered significant. As an additional control, sera from 150 patients selected at random from the Arthritis Clinic were also tested.

Results. Isolation attempts were successful in 5 of the 8 patients tested. In these attempts the eggs began dying in the second passage. After several passages the isolates had titers in excess of 10^7 ELD 50/g of yolk sac and were positive for group antigen at dilutions greater than 1:128. Bedsoniae were isolated from all 4 of the patients from whom synovial material was obtained and from the urethra and conjunctiva of a fifth patient (Table I). The sera of 3 of these 5 patients had CF titers against the psittacosis antigen of 1:16 or greater. In addition, 2 of the patients with Reiter's syndrome, on whom only serologic tests were done, had CF titers in excess of 1:16.

Bedsoniae were not isolated from the synovial fluid or membranes of the 15 patients with osteoarthritis or rheumatoid arthritis. Serum CF titers against the psittacosis antigen were insignificant in these patients. Of the 150 patients selected at random from the Arthritis Clinic, only 2 had serum CF titers as high as 1:16.

In 2 cases particles resembling elementary

bodies were seen in Giemsa-stained preparations. Several specimens appeared to show a positive reaction to fluorescent antibody staining. None of the test specimens contained perinuclear inclusions, such as are found in trachoma and inclusion conjunctivitis.

Inoculation of the isolates (by dropping) into the conjunctival sac of several species of susceptible monkeys did not result in the follicular conjunctivitis characteristically produced by the agents of trachoma and inclusion conjunctivitis. Furthermore, the latter agents are virulent in the mouse only when injected intravenously, whereas the bedsoniae isolated in this study were pathogenic when given by the intracerebral, intraperitoneal and intranasal routes, as well as by intravenous injection. The bedsoniae isolates also could be propagated serially in L or HeLa cells. Clear-cut differentiation of our isolates from the agent of LGV is more difficult. The fresh isolates do not possess the pathogenicity patterns of LGV strains; the validity of identifying strains by this means, however, is open to question.

Discussion. The isolation of bedsoniae from the synovial fluid or membranes of 4 patients and the significant CF titers of sera from 5 of the 16 patients studied lend support to the hypothesis that these agents may play a role in the arthritis of Reiter's syndrome. In all cases in which positive results were obtained, the patients were studied during the initial attack of the disease. The successful isolation attempts span the period from September 1964 to December 1965. None of

these patients has shown evidence of relapse during this interval.

The possibility that the bedsoniae isolated in this study were the agents of trachoma or inclusion conjunctivitis appears to be ruled out on several counts. Exclusion of the agent of LGV was less certain. Several reports of mycoplasma involvement in Reiter's syndrome have not established the role of these organisms in the disease(7). As suggested in a previous report(3), Reiter's syndrome may represent a symptom-complex. As such, several etiologic agents might produce similar clinical manifestations. Alternatively, the provocation of the first infection may set up fertile ground for repeated infection by other agents. Thus, mycoplasmas or bedsoniae might be the causative agents, individually or conjointly. Once the agent or agents have been isolated, these conjectures should be amenable to experimental investigation. Although we have not attempted mycoplasma isolations, the possibility of their involvement in the present cases cannot be ignored.

In the future, attempts to isolate bedsonia and mycoplasma organisms from patients with various types of arthritis, including Reiter's syndrome, will be carried out. Currently, attempts are being made to produce arthritis by injection of the isolates into animals. In preliminary studies, intraperitoneal injection of yolk-sac suspensions of the isolates in

guinea pigs has produced arthritis in some instances. Of greater significance, arthritis has been regularly produced by intra-articular injection of the isolate into subhuman primates. These findings will be reported later.

Summary. Studies in 16 patients with Reiter's syndrome, consisting of arthritis, urethritis and/or conjunctivitis, suggest that bedsoniae (psittacosis-lymphogranuloma venereum-trachoma group of microorganisms) may play a role in arthritis in man. Attempts to isolate bedsoniae from synovial material or urethral and conjunctival scrapings from 8 patients were successful in 5 instances. Five of the 16 patients had significant complement-fixation titers against a psittacosis group antigen.

1. Mendlowski, B., Segre, D., *Am. J. Vet. Res.*, 1960, v21, 68.

2. Siboulet, A., Galistin, P., *Brit. J. Ven. Dis.*, 1962, v38, 209.

3. Bauer, W., Engleman, E. P., *Trans. Assn. Am. Phys.*, 1942, v307, 57.

4. Jones, B. R., *Brit. J. Ven. Dis.*, 1964, v40, 3.

5. Meyer, K. F., Eddie, B., in *Diagnostic Procedures for Viral and Rickettsial Diseases*, 2nd ed., Am. Public Health Assn., New York, 1965, p399.

6. Schachter, J., Doctoral Dissertation, Univ. of California, Berkeley, 1965.

7. Channock, R. M., *New Engl. J. Med.*, 1965, v273, 1199.

Received February 8, 1966. P.S.E.B.M., 1966, v122.

Acute Effect of Metal Ions on Uptake of Alpha-Aminoisobutyric Acid (AIB) by Rat Parathyroids *in vivo*.* (31114)

WILLIAM Y. W. AU, JUDITH R. ENGERMAN, AND LAWRENCE G. RAISZ†
(Introduced by Harold C. Hodge)

Departments of Pharmacology and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

In a previous study(1), we demonstrated that the rat parathyroid responds to chronic hypocalcemia by an increase in size and in ability to concentrate amino acids as meas-

* This study was supported by Grants AM 08205 and AM 07871 from USPHS.

† Burroughs Wellcome Scholar in Clinical Pharmacology.

ured by the distribution ratio of the non-metabolized amino acid analogue, alpha-amino isobutyric acid (AIB). In short-term experiments *in vitro* the uptake of AIB and the uptake and incorporation into proteins of several natural amino acids by the parathyroid were found to be inversely related to the calcium concentration of the medium(2). In