SERO-NEGATIVE POLYARTHRITIS THE *BEDSONIA* (*CHLAMYDIA**) GROUP OF AGENTS AND REITER'S DISEASE

A PROGRESS REPORT†

BY

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This Medical Research Council (MRC) Group has studied infection at various sites, and carried out tests for the *Bedsoniae*, in selected groups of patients, with the object of determining the relationship of genital infection to disease of the eyes and of joints. We use the name *Bedsonia* (or *Chlamydia*) for any member of the psittacosis-lymphogranuloma venereum-TRIC group of agents. The term TRIC is used (Gear, Gordon, Jones, and Bell, 1963) for an agent recovered from the eye of a patient suffering from trachoma or inclusion conjunctivitis, the TR-being derived from trachoma and the -IC from inclusion conjunctivitis. The term TRIC agent can be used for an isolate obtained from a site other than the eye only if that isolate is shown to have the same characteristics, including pathogenicity for the eye, as TRIC agent. We use the term "agent" rather than "virus" (Harper, 1966) because the *Bedsoniae* are not viruses: thus they contain both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), they reproduce by binary fission, contain cell wall constituents similar to those of bacteria, and possess enzyme systems.

Findings have been reported in a series of publications (Al-Hussaini, Jones, and Dunlop, 1964; Dunlop, Jones, and Al-Hussaini, 1964; Jones, Al-Hussaini, and Dunlop, 1964; Al-Hussaini, Jones, and Dunlop, 1965; Dunlop, Al-Hussaini, Garland, Treharne, Harper, and Jones, 1965a; Dunlop, Jones, and Al-Hussaini, 1965b; Jones, Al-Hussaini, and Dunlop, 1965; Dunlop, Harper, Al-Hussaini, Garland, Treharne, Wright, and Jones, 1966a; Dunlop, Al-Hussaini, Freedman, Garland, Harper, Jones,

Race, du Toit, Treharne, and Wright, 1966b; Freedman, Al-Hussaini, Dunlop, Emarah, Garland, Harper, Jones, Race, du Toit, Treharne, and Wright, 1966; Harper, Al-Hussaini, Dunlop, Emarah, Freedman, Garland, Jones, Race, du Toit, Treharne, and Wright, 1966; Jones, Al-Hussaini, Dunlop, Emarah, Freedman, Garland, Harper, Race, du Toit, and Treharne, 1966) and the literature has been briefly reviewed (Jones, 1964; Dunlop, 1965; Harper, 1966).

With regard to Reiter's disease, Dunham, Rock, and Belt (1947) reported the presence of an unidentified agent after passage in eggs of material from the urethra and from the eye of a patient. Siboulet and Galistin (1962) obtained isolates from urethral and conjunctival material from three patients. The isolates were not maintained and so were not fully identified. Schachter, Barnes, Jones, Engleman, and Meyer (1966) reported isolations of *Bedsoniae* from synovial fluid and membrane, urethra, and conjunctiva, in a study of eight patients. The first isolate of a *Bedsonia* that we obtained from eggs inoculated with material from a joint was reported in 1966 (Dunlop and others, 1966b; Harper and others, 1966).

There is evidence to suggest that, although the *Bedsoniae* may be difficult to culture, they may be more resistant to adverse conditions than had been suspected in the past. Thus Sowa, Sowa, Collier, and Blyth (1965) recovered agent after it had been kept on cotton cloth for 90 minutes at room temperature. In experiments in our laboratory, Darougar (1967) dipped rubber teats into 10 per cent. and 20 per cent. suspensions of infected yolk sac and allowed them to dry. He recovered a *Bedsonia* from some rubber teats after they had remained for no less than 26 hours and 40 minutes at room temperature after drying. It is clear that

*The term *Chlamydia* has been re-proposed as the correct name for this group (Page, 1966).

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agents as resistant as this may be a source of cross-contamination in the laboratory and of infection to laboratory staff.

A study of the techniques that we have used in our laboratory (Harper, Dwyer, Garland, Jones, Treharne, Dunlop, Freedman, and Race, 1967) has shown that they may have permitted spurious isolation: thus, *Bedsoniae* were isolated after the passage of yolk-sac material from eggs inoculated with sterile sucrose potassium glutamate. Infection seems to have entered during the transfer of material from one series of eggs to the next. There appears to have been little opportunity for contamination to occur at the initial inoculation of eggs.

In view of these findings we reviewed our work, discarding, as evidence of infection, all isolates obtained later than the first passage. The results were presented at the Conference on these agents, organized by the World Health Organization, that was held in California in the autumn of 1966 (Dunlop, Freedman, Garland, Harper, Jones, Race, du Toit, and Treharne, 1967; Harper and others, 1967).

Methods

The techniques used in the study of the genital tract have already been outlined (Dunlop and others, 1964; Dunlop and others, 1965a). Briefly, the essentials were:

(1) With regard to tests for *Bedsoniae*, to obtain as high a yield as practicable of superficial epithelial cells for testing from the urethra in the male, and from the marginal area of the cervix and from the rectum in the adult female;

(2) To diagnose any inflammatory change or sexually-transmitted infection, if present;

(3) To study and to record the appearances in the genital tract (and the rectum in the cases of women) with a Zeiss operating microscope using magnifications similar to those used for inspection of the eye.

Joint fluids were transported in sterile universal glass bottles containing 0.3 ml. of 20 per cent. sodium citrate, usually without added antibiotics.

Conjunctival scrapings were tested if there was any clinical evidence of inflammation of the eye.

Eggs were inoculated as soon as possible, and always within 3 hours of the specimen having been obtained. Specimens sent for culture from centres other than the Institute of Ophthalmology were packed in ice for transport to the laboratory.

Patients

We here present the results of tests of two groups of patients:

(1) Twenty patients suffering from polyarthritis probably due to Reiter's disease whom we have examined;

in no case was a positive result obtained from Latex tests for rheumatoid factor. The sexual contacts of these patients were also examined if possible.

In the cases of patients with "sero-negative" polyarthritis, a first essential is to establish whether evidence of genital infection is present. In fourteen of the nineteen men, urethritis was a marked and obvious feature; in the remaining five, genital inflammation was inconspicuous and could be diagnosed only as a result of examination of smears of the overnight urethral secretion, first part of the overnight urine, or of the fluid expressed by massage of the prostate and seminal vesicles. The one woman was found to have proctitis and cervicitis.

Thirteen of the twenty patients were suffering from clinical acute Reiter's disease in which at least three components of the syndrome had developed; these thirteen men were all suffering from acute urethritis and polyarthritis and from at least one of the following: papillary conjunctivitis, characteristic changes of genital or oral mucous membranes or both, keratoderma.

The remaining seven of the twenty patients were suffering from genital infection and polyarthritis, with additional iritis in two cases. Clinical diagnosis was less certain in this group. Two of the seven had psoriasis; in two other cases, but for the evidence of genital infection, ankylosing spondylitis would have been accepted as the likely diagnosis.

In all twenty cases disease was active at the time of examination.

(2) In the United Kingdom Reiter's disease occurs mainly as a complication of so-called non-specific urethritis ("NSU"). Hence the second group consists of 89 men who presented because of untreated "NSU"; the female sexual contacts of these patients were also examined if possible.

Results

With regard to Reiter's disease: genital tests have been performed in twenty cases in which that diagnosis was likely, including that of one woman (Table I). There was genital inflammation in all and isolates were apparently obtained from the urethra of one man (Mr. HY) and from the cervix of the one female patient (Mrs. AC), who presented because of trachoma. She also had psoriasis which can produce a pattern of arthropathy indistinguishable from that of Reiter's disease.

TABLE I
PROBABLE REITER'S DISEASE: RESULTS OF TESTS FOR *BEDSONIAE*

Tests	No. of patients	Isolate obtained*
Genital	20	2
Synovial	8	4†

*No first-pass isolate.

†From synovial fluid—three patients.

From synovial membrane—one patient.

Synovial material from eight patients has been tested and isolates have apparently been obtained from four: thus isolates were apparently obtained from synovial fluid from two separate specimens in each of two cases of men suffering from classical Reiter's disease (Mr. IE, Mr. IL): an isolate was apparently obtained from synovial fluid from a knee of a third patient (Mr. GH) who was suffering from polyarthritis, psoriasis, and low-grade genital inflammation. This isolation was reported at the meeting of the Ophthalmological Society of the United Kingdom in April, 1966 (Dunlop and others, 1966b; Harper and others, 1966). Siboulet (1965) had previously tested this patient and found inclusions in urethral and conjunctival scrapings and had isolated an agent from urethral material; growth of this agent was not maintained so it was not available for comparison with our isolate from a joint: a specimen of synovial fluid was examined with negative result from a fourth patient (Mr. HE) who was probably suffering from Reiter's disease; 3 months later synovectomy of the left knee was carried out. Cellular changes in the synovial fluid suggesting infection by a member of the *Bedsonia* group were then present but culture gave negative results. Scrapings from the excised synovial tissue were also cultured with and without added streptomycin and neomycin: a member of the *Bedsonia* group was isolated at the second passage from the specimen without added antibiotics. Examination of the eye of this Egyptian patient showed signs of long-standing trachoma which presumably dated from early life. Tests of the conjunctiva gave negative results.

Eleven sexual contacts of these patients have been tested. Isolates were apparently obtained from both the cervix and rectum in one case (Mrs. FF) and from the cervix in another (Miss HY).

No isolate was obtained at first passage from any patient suffering from Reiter's disease or from any sexual contact of such a patient. Inclusions were found only in the conjunctival scrapings from the one female patient (Mrs. AC) who was suffering from trachoma, psoriasis, genital inflammation with cervicitis, proctitis, and polyarthritis. From the material that we have studied then there is no certain proof that infection with the *Bedsoniae* is related to Reiter's disease.

Schachter and his colleagues are satisfied that the *Bedsoniae* that they have obtained from joints affected by Reiter's disease are true isolates (Schachter, 1967b). When tested in the laboratory, the first isolate we obtained from eggs inoculated with material from a joint of one of our patients (Mr.

GH) closely resembled the isolates that Schachter and others have since described. No agent of this nature had been introduced into our laboratory from any other centre.

In the United Kingdom, Reiter's disease in a great majority of cases is a complication of so-called non-specific urethritis (NSU). Accordingly, men presenting because of that condition have been studied and evidence of infection has been found in some cases in them and in their contacts. Table II shows that we have now tested urethral material for members of the *Bedsonia* group from 89 men presenting because of untreated "non-specific" urethritis. A link between this condition and Reiter's disease is shown by the fact that one of these patients went on to develop Reiter's disease and is considered also with that group.

TABLE II
RESULTS OF TESTS: 89 MEN PRESENTING BECAUSE OF
"NON-SPECIFIC" URETHRITIS ("NSU")

Urethra: Isolate obtained	15 ¹	} 22
Isolate + Inclusion	4 ²	
Inclusion found	3	

¹ = First pass in one case.

² = First pass in three cases.

In 22 of the 89 cases of "non-specific urethritis" (or one-quarter) tests gave positive results. In each of fifteen cases an isolate was apparently obtained without accompanying inclusions; in one of these (Mr. BW) the isolate was obtained at the first passage. In each of four cases an isolate was apparently obtained and an inclusion was found; in three of these cases (Mr. AY, Mr. CO, Mr. FW) the isolates were obtained at the first passages. Inclusions were found in three cases. If isolates obtained after the first passage are excluded, then the number of cases with positive findings is reduced from 22 to eight.

The female sexual contacts of some of the men suffering from "NSU" were examined (Table III).

TABLE III
RESULTS OF TESTS: 40 FEMALE CONTACTS OF "NSU"

History of vaginal discharge	20
Cervix: "Follicles"	18
Proctitis	11
Cervix: Isolate obtained	15
Inclusion found	1 ²
Rectum: Isolate obtained	4
Associated infection	8

"NSU" = "Non-specific" urethritis.

² = First pass in two cases.

Twenty of forty gave a history of vaginal discharge. Appearances of the marginal area of the cervix were seen resembling those produced by TRIC agent in the conjunctiva, thus "follicles" were present in 18 cases. There was proctitis in eleven. Isolates were apparently obtained in fourteen cases and an inclusion in one more, so that tests of the cervix gave positive results in fifteen of the forty cases. In the cases of two patients (Mrs. GO, Mrs. BM), the cervical isolates were obtained at the first passages; in one more an inclusion was found. If isolates obtained after the first passage are excluded, then the number of cases with positive findings is reduced from fifteen to three. In each of four cases isolates were apparently obtained from both cervix and rectum. Associated infection was present in eight cases, namely trichomoniasis in five with candidiasis in one, and candidiasis alone in three. If it is accepted that "non-specific" genital infection is sexually transmitted, it is not surprising that other sexually-transmitted disease is found in association with it.

The Figure shows appearances of the marginal area of the cervix resembling follicles in the case of a contact (Mrs. EL) of a patient who presented because of "non-specific" urethritis. Similar findings in the cases of mothers of babies suffering from TRIC ophthalmia neonatorum and in cases of women suffering from ocular infection due to TRIC agent have been published previously.

We have also tested indirectly for this group of agents by carrying out tests for complement-fixing antibody to the *Bedsoniae* (LGVCF) and intradermal tests (Barwell, Dunlop, and Race, 1967).

Results have been disappointing: with regard to the LGVCF only about one-quarter of the mothers of babies infected with TRIC agent produced significant levels of fixation and another quarter showed no fixation at all. Results of intradermal tests were even less sensitive. Judging by this rather

Figure.—Cervix (Mrs. EL) showing "follicles" in marginal area.
× 11 approx.
F = "follicle"

special group, we can expect these indirect tests to give negative results in a high proportion of infected persons. In a total of eleven cases in patients and contacts in the "NSU" group first-passage isolates were obtained, inclusions found, or both. In these eleven cases of proven infection, the LGVCF showed complete fixation to 1 in 32 in only two, lower levels of fixation in six, and negative results in three.

Table IV shows the results of tests in the probable Reiter's disease group in the cases of patients and consorts from whom isolates were reported. There was no significant fixation in the LGVCF, which differs from the experience of Schachter and his collaborators (Schachter and others, 1966; Schachter, 1967a). The Frei test was positive in two cases, an intradermal test with an acid-extract of LGV was positive in four cases, and a similar so-called specific

TABLE IV
PROBABLE REITER'S DISEASE:
RESULTS OF TESTS IN PATIENTS AND CONSORTS WITH POSITIVE TESTS FOR *BEDSONIAE*

Subjects		Source of Isolate*	Fixation in LGVCF	Frei	LGV Acid-extract	TRIC Acid-extract
Patients	Mr. HY	Urethra	0	—	—	—
	Mr. GH§	Joint	0	+	+	+
	Mr. IE	Joint	0	—	+	+
	Mr. IL	Joints	0	—	+	+
	Mr. HE†	Joint	0	—	+	+
	Mrs. AC‡	Cervix (also conjunctival inclusion)	Complete at 1 in 4	—	—	—
Consorts	Mrs. FF	Cervix and Rectum	Partial at 1 in 8	+	+	—
	Miss HY	Cervix	0	—	—	—

*No first-pass isolate

†Also trachomatous scarring

‡Also trachoma and psoriasis

§Also psoriasis

extract of TRIC agent gave positive results in three cases. Thus, intradermal tests were positive in three of the four cases in which isolates were apparently obtained from joints.

Discussion

In considering the results that we have presented, we should first take into account the insensitivity of the tests we have employed for TRIC agent and other members of the *Bedsonia* group. Even if we discount the possibility of spurious isolation, the evidence suggests that these tests are certainly insensitive for material from the genital tract. Thus, in a study of 28 mothers (Dunlop and others, 1967), all of whom had shown that they were infected by giving birth to babies suffering from ophthalmia neonatorum due to TRIC agent, initial tests detected TRIC agent in only six cases, or approximately one in five. In two further cases in which clinical signs in the genital tract persisted or recurred despite treatment, subsequent tests eventually gave positive results. In this same series it is likely that all the fathers who were found to be suffering from urethritis were infected with TRIC agent, and yet tests of the urethra apparently confirmed this in only seven of the seventeen such cases. This suggests that the true incidence of infection of the genital tract by TRIC agent and by other *Bedsoniae* could be higher than suggested by this study.

In some cases of Reiter's disease, the diagnosis is very obvious with gross genital infection, typical polyarthritis, keratoderma, lesions of genital and oral mucous membranes, and conjunctivitis. In others, as has been implied, the diagnosis is much less clear: the pattern of joint involvement may suggest the disease but genital infection may not be clinically obvious.

Differentiation between some cases of Reiter's disease and ankylosing spondylitis and between Reiter's disease and psoriatic arthropathy, as pointed out by Wright and Reed (1964), may be more than difficult. In some cases distinction may be artificial: perhaps, for example, when an individual with a tendency to psoriasis acquires genital infection he is more likely to develop polyarthritis. The finding by Lawrence (1964) that the relatives of patients suffering from Reiter's disease have an increased incidence of psoriasis seems relevant here.

Accordingly, we have presented together the results of tests in twenty cases of polyarthritis in which "non-specific" genital inflammation was also present. The term "probable Reiter's disease" is used for this group. With the small numbers

involved at this stage further subdivision does not appear profitable.

When "non-specific" genital infection is detected, however, this finding may be coincidental because such infection is widespread in the community. The way to prove a connexion would be to isolate a pathogenic agent both from the genital tract and from an affected joint and to show that the agents from different sites were identical. This is the object we are pursuing.

It does not seem likely to us that an "agent of Reiter's disease" will be found as the sole cause of the disease. If this were the case, infection from a patient suffering from Reiter's disease would produce Reiter's disease. If this happens it is a rare event. It seems more likely that agents cause infection and that the occasional individual reacts to that infection by producing Reiter's disease as a complication. As already noted, in our "non-specific" urethritis series, one of the 89 men went on to develop Reiter's disease. The link between "non-specific" urethritis and Reiter's disease is further shown by the fact that the husband of a sexual partner of another of these men suffering from "NSU" was admitted to hospital elsewhere because of severe Reiter's disease. The converse was seen in our series of patients presenting because of Reiter's disease: here a second male contact of the girl-friend of a man suffering from Reiter's disease was examined and was found to have "non-specific" genital infection.

In our future studies we shall examine further patients in the same groups as before, using laboratory procedures that have been carefully controlled to obviate the possibility of spurious isolation in so far as this can be done. While the careful assessment of clinical material is obviously fundamental, it is also clear that a most exacting control of laboratory procedures is essential. Attempts to increase the sensitivity of tests will be made and, if they are successful, the results of examination in control cases will have more meaning.

Summary

Results of clinical examination of the genital tract and of laboratory tests are reported in two groups of patients: twenty patients probably suffering from Reiter's disease, and eleven of their sexual contacts; 89 men presenting because of untreated "non-specific" urethritis, and forty of their sexual contacts.

Spurious isolation of members of the *Bedsonia* group has occurred in studies of laboratory technique. Results of investigations obtained so far are reconsidered in the light of these findings.

We are grateful to our colleagues who have referred patients to us for investigation; to Dr. A. E. Wilkinson, director of the VD Reference Laboratory of The London Hospital, for carrying out serological tests for syphilis and

gonorrhoea, and cultures for bacteria, *Trichomonas vaginalis*, and *Candida*; to the Department of Medical Illustration, Institute of Ophthalmology, for preparing the illustration.

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Polyarthrite séronégative

Les agents du groupe *Bedsonia* (*Chlamydia*) et la maladie de Reiter

RÉSUMÉ

On rapporte les résultats des examens cliniques de l'appareil génital et des épreuves de laboratoire chez deux groupes de malades: le premier groupe comprenait 20 malades atteints probablement de maladie de Reiter et onze personnes qui avaient eu des rapports sexuels avec eux, le deuxième groupe incluait 89 hommes ayant consulté pour une uréthrite "non spécifique" et non traitée et 40 personnes ayant eu des rapports sexuels avec eux.

Au cours d'étude des méthodes de laboratoire il est arrivé qu'on isole des faux microorganismes ressemblant aux agents du groupe *Bedsonia*. A la lumière de cela on reconsidère les résultats des recherches faites jusqu'à ce jour.

Poliarthritis seronegativa

Los agentes del grupo *Bedsonia* (*Chlamydia*) y la enfermedad de Reiter

SUMARIO

Se relatan los resultados de la investigación clínica y de laboratorio del aparato genital en dos grupos de enfermos: uno de 20 enfermos con probable enfermedad de Reiter y de 11 personas que tuvieron relación sexual con ellos; el otro de 89 hombres con uretritis no específica y no tratada y de 40 personas que tuvieron relación sexual con ellos.

Espurio aislamiento de miembros del grupo *Bedsonia* ha ocurrido en los estudios del procedimiento de laboratorio. Se vuelven a considerar los resultados de las investigaciones hasta la fecha en la luz de este hallazgo.