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A NOTE ON THE RESULTS OF CULTURAL AND SEROLOGICAL TESTS FOR PLEUROPNEUMONIA-LIKE ORGANISMS IN REITER'S DISEASE*

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The isolation of pleuropneumonia-like organisms (PPLO) from the urethral secretions of patients with non-gonococcal urethritis has been reported by many workers and has led to the suggestion that they may be a possible cause of this condition, although they have also been found in the genital tract of apparently healthy males. Other organisms of the pleuropneumonia group are known to be associated with genital infection in animals, and the frequency of joint lesions in infections with some rat strains has led to the search for PPLO in cases of Reiter's disease, especially as Dienes, Ropes, Smith, Madoff, and Bauer (1948) have reported the isolation of PPLO from synovial fluid obtained from an affected joint in this condition. Subsequent work has not lent support to this view, and Kleineberger-Nobel (1959b) grew PPLO from the urethra of only two out of twenty patients with Reiter's disease and from none of the specimens of joint fluid examined.

Because further information seemed desirable, patients with Reiter's disease attending The London Hospital have been examined by cultural and serological methods for evidence of the presence of PPLO and an attempt has been made to relate the bacteriological findings to the stage of the disease and to the effects of treatment.

Material and Methods

Two groups of patients were examined:

(*a*) Those who attended The London Hospital with an attack of Reiter's disease during the period of study.

(*b*) Those who had suffered one or more attacks in the past were asked to re-attend for examination. It was felt that, because recurrences are so common in this condition, investigation of this group would be of value.

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The diagnosis of Reiter's disease was made only on the evidence of a personally-observed attack of arthritis associated with urethritis. The patients were classified as having *active* disease if non-specific genital infection was present at the time of examination with clinical evidence either of arthritis of one or more joints or of active iridocyclitis; only one patient in the series fell into the second category.

Where urethritis was present, the urethral secretion and a centrifuged deposit of urine were cultured for PPLO. When there was no discharge, as in clinically inactive patients, prostatic massage was performed and the prostatic fluid cultured. Samples of blood were taken from all patients and complement-fixation tests were performed with two antigens, one made from a strain of PPLO isolated from a case of non-gonococcal urethritis (NGU antigen) and a second from a strain isolated from a case of Reiter's disease (R. antigen).

Cultures were made on the medium described by Klieneberger-Nobel (1959a) and the plate was incubated at 37°C. in a moist atmosphere for one week before being discarded as negative. PPLO were identified by their colonial appearance and were checked where necessary by the staining method described by Dienes and others (1948). Subcultures were made to a similar medium without penicillin to avoid possible confusion with bacteria in their L phase.

Antigens for complement-fixation tests were prepared by the method described by Card (1959), only unheated suspensions being used. The optimal titre for each antigen (usually 1 in 10) was determined by an optimal proportions titration. The complement-fixation technique was based on the Whitechapel Wassermann method (Price, 1949; 1950), except that a dose of 1·3 M.H.D complement was used in the test and incubation was carried out overnight in the refrigerator followed by 30 minutes at 37°C. before

the addition of sensitized cells, the tests being read after a further 30 minutes at 37°C. In the screen test the final serum concentration in the reaction mixture was 1 in 16 (0·022 ml. serum, 0·11 ml. saline, 0·11 ml. antigen, and 0·11 ml. complement). Sera giving positive reactions were re-tested quantitatively. Veronal-buffered saline with added Ca++ and Mg++ was used throughout as a diluent. As the minimal amount of complement was used in the test to enhance sensitivity, no positive result was accepted as valid unless the control tube showed complete lysis.

Results

During the course of the investigation 85 patients were examined, but 31 of these were rejected either because they had not been personally examined or because they did not satisfy the diagnostic criteria described. Of the remaining 54, 31 were active and 23 inactive; Tables I–IV record the results of tests on these two groups.

TABLE I

RESULTS OF CULTURES AND COMPLEMENT-FIXATION TESTS ON 31 PATIENTS WITH *ACTIVE* REITER'S DISEASE

Test	Results	No. of Patients		Total
		Untreated*	Treated*	
Culture ..	Positive	9	0	31
	Negative	10	7	
	Not done	5		
Complement-Fixation Test	Positive	13	3	31
	Negative	11	4	

* At the time specimens were taken for examination, see Discussion.

TABLE II

COMPARISON OF CULTURE AND COMPLEMENT-FIXATION TEST RESULTS ON 31 PATIENTS WITH *ACTIVE* REITER'S DISEASE

Test	Results				Total
Culture	+	+	0*	0	31
Complement-Fixation Test	+	0	+	0	
No. of Patients	5	4	11	11	

* No cultures made in five cases.

TABLE III

COMPARISON OF RESULTS GIVEN BY TWO ANTIGENS IN TESTS ON 31 PATIENTS WITH *ACTIVE* REITER'S DISEASE

Antigen	Results				Total
NGU	+	+	0	0	31
R	+	0	+	0	
No. of Patients ..	10	1	5	15	

TABLE IV

RESULTS OF TESTS ON 23 PATIENTS WITH *INACTIVE* REITER'S DISEASE

No. of Patients in Group*	23
No. of Cultures Positive	0
No. of Complement-Fixation Tests Positive (both antigens)	2

* Cultures or complement-fixation tests were not performed in two cases.

Specimens were taken from seven of the patients with active disease only after treatment had been given. No PPLO were grown and complement-fixing antibody was detected in only three of these sera, the R antigen giving low titred results while tests with the NGU antigen were negative.

Serial CFTs were performed on four patients in the active group who were initially found to be sero-negative, and the subsequent tests were also negative. One patient with active disease, whose culture and CFT were both positive, was treated with tetracycline by mouth; a subsequent culture and CFT were negative and this coincided with a clinical improvement in the patient's condition.

All the patients in the "inactive" group had received treatment with an antibiotic which was effective *in vitro* against PPLO during the acute stage of their illness.

Discussion

PPLO are not commonly found in the genital tract of normal males, especially below the age of puberty. Kleineberger-Nobel (1959b) reported their isolation from the urine of only three out of 100 presumably healthy adults (one of these three was shown to have non-gonococcal urethritis) and from none of 47 boys under 13 years of age. Our own results are similar, no PPLO having been grown from the urines of 100 boys under 12 years of age and from only five out of 51 young male medical students. In contrast, PPLO were grown from the urethral secretions of nine out of 26 males with active Reiter's disease; seven of the patients whose cultures were negative had been treated with drugs active against PPLO *in vitro* before the cultures were taken, so that the number of isolations achieved may underestimate the true incidence. No PPLO were grown from patients with inactive Reiter's disease who had been treated in the past. It should be noted in this context that Kleineberger-Nobel (1959b) was able to culture PPLO from 48 per cent. of 65 cases of non-gonococcal urethritis and from 30 per cent. of 49 male cases of gonococcal urethritis.

The strains of PPLO isolated from patients with Reiter's disease during the present investigation did not show any morphological or cultural differences from those which we have isolated from patients with either gonorrhoea or non-gonococcal urethritis.

The significance of a positive complement-fixation test for PPLO is still uncertain. Stokes (1955) and Melén and Gotthardson (1955) reported finding complement-fixing antibodies, often to high titre, in sera from women with genital infections. Card (1959), on the basis of an extensive survey, thinks it likely that the antibody is the result of some previous infection with human genital PPLO. She found only two sera giving positive reactions in 104 children under 13 years of age, and in two out of 188 male blood donors, in contrast to eighteen out of 122 patients with non-gonococcal urethritis. Our own preliminary results have shown a higher incidence of positive reactions, which was possibly due to differences in the technique used. Three positive results were found in tests on sera from 62 children between the ages of 3 months and 12 years and in 14 per cent. of 93 male blood donors. These figures refer to the NGU antigen. This gave eleven positive results, usually only to a low titre (1 in 4 or 8), with 31 sera from patients with active Reiter's disease but only two positive results with 23 patients in whom the condition was inactive. Card found only one positive result with nineteen sera tested, but does not mention the clinical status of the patients at the time of examination. As shown in Table III, the antigen made from a strain of PPLO isolated from a case of Reiter's disease appeared slightly more sensitive than that made from an NGU strain, but the differences in titre were not marked. Card states that, while human genital strains of PPLO form a serological group distinct from the human mouth strains and from animal strains, this group is not quite antigenically homogeneous.

Although the number of patients examined so far is small, the high incidence of positive cultures or complement-fixation tests for PPLO in patients with *active* Reiter's disease suggests that the possibility that PPLO play a part in the causation of the condition cannot yet be excluded.

Summary

(1) Cultures and serological tests for PPLO were carried out on 54 patients with Reiter's disease.

(2) Positive cultures were found in nine and positive complement-fixation tests in sixteen out of 31 patients with clinically active disease. No positive cultures and only two positive complement-fixation tests were found in 23 males with a past history of the condition which was inactive at the time of examination.

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