MICROBIOLOGICAL STUDIES OF REITER'S DISEASE*†

BY

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In spite of increasing interest in Reiter's disease, its cause remains unknown. The earliest reported cases often are thought to date from the first world war, but it was undoubtedly this disease which Brodie (1818) described in his excellent case reports, and it is interesting that he called attention to features which led him to suspect an infectious, venereal origin. During the first world war, Fiessinger and Leroy (1916-17) in a detailed report of an epidemic of dysentery among French troops on the Somme Front in 1916, described four cases of what they called the *syndrome conjunctivo-urethro-synoviale* to a meeting of the Société Médicale des Hôpitaux de Paris. Finding no evidence of gonococcal infection, they naturally concluded that the syndrome was due to infection with *Shigella dysenteriae*, although they were unable to isolate this or any other micro-organism from urethral or conjunctival exudates or synovial fluid. Six days later Reiter's well-known case report of a young German officer was published; he erroneously concluded that the cause was a spirochaete which he found in the blood of this patient (Reiter, 1916). It is noteworthy that the illness in Reiter's patient also began with severe diarrhoea, and in the more recent literature many additional cases of Reiter's disease occurring in patients with bacillary dysentery have been reported (Young and McEwen, 1947; Paronen, 1948; Fowler and Knight, 1956; Bailey and Bishop, 1959). Nevertheless, most cases appear to be of venereal origin.

A new approach to the problem was introduced by Dienes and Edsall (1937), when they first demonstrated the presence of PPLO in humans and later showed that the intranasal instillation of pathological material from patients suffering from rheumatic fever and rheumatoid arthritis induced pleuro-

pneumonia in mice (Sullivan and Dienes, 1939). These workers did not believe that the PPLO came from the human tissues, but subsequently the role of PPLO in rheumatic diseases and in the related question of non-gonococcal urethritis was conjectured by several authors (Dienes, 1940; Sabin and Johnson, 1940; Dienes and Smith, 1942; Beveridge, 1943; Beveridge, Campbell, and Lind, 1946; Salaman, King, Bell, Wilkinson, Gallagher, Kirk, Howarth, and Keppich, 1946; Wallerstein, Vallee, and Turner, 1946; Williams, 1946; Dienes, Ropes, Smith, Madoff, and Bauer, 1948). There had, however, been no report of isolation of PPLO from diseased joints until Dienes and others (1948) described two instances in which PPLO were obtained from the synovial fluid of a patient with Reiter's disease. Since that date more than 300 papers have dealt with the role of PPLO in rheumatic conditions and in the pathogenicity of non-gonococcal urethritis. [A complete compilation of the literature has been made by Reiter (1960) and a critical evaluation of the question is given by Freundt (1958) and Klieneberger-Nobel (1962).] In spite of this, no clear answer has been obtained. It has, however, been clearly established that, whereas PPLO can rarely be cultured from the genital tract of prepubertal males, they occur not infrequently in normal women and are common in both males and females with a variety of genito-urinary disorders. In recent years, also, a possible virus aetiology of Reiter's disease has been conjectured (Harkness, 1945, 1947; Baines, 1947; Coutts, 1947; Short, 1947; Dunham, Rock, and Bell, 1947; Hall and Finegold, 1953; Robinson and 11 others, 1953; Willcox, 1957; Mavroglu, 1958; Burgess, 1959). Visualization of inclusion bodies (Harkness, 1945, 1947) or isolation of viruses (Baines, 1947; Dunham and others, 1947; Robinson and others, 1953) have been claimed in some reports, but Ford (1956, 1958) obtained only negative results.

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The question of causation has also been confused by diagnostic deficiencies. For many years, for example, clinicians did not distinguish between gonococcal arthritis and Reiter's disease, and the latter was thought to be either ordinary gonococcal arthritis in an attenuated form or perhaps an allergic synovial response to products of the gonococcus. Indeed, the latter view is still held by some investigators (Weinberger and Bauer, 1955; Csonka, 1959a, b), who suggest that the relationship of Reiter's disease to gonococcal arthritis is analogous to that of rheumatic fever to haemolytic streptococcal suppurative arthritis. It might be conjectured today that, if the mechanism is an allergic one, it is of delayed type and may, perhaps, be induced by such micro-organisms as *Shigella dysenteriae* as well as by *Neisseria gonorrhoeae* (Twiss and Douglas, 1946; Warthin, 1948; Ogryzlo and Graham, 1950; Hall and Finegold, 1953).

The present study is part of a broad clinical, pathological, serological, and microbiological investigation of Reiter's disease and others of the less clearly defined forms of diseases affecting the joints, which have claimed the interest of the New York University Rheumatic Diseases Study Group in recent years. In this paper the essentially negative results of our attempts to isolate viruses and PPLO will be reported.

Methods

Material.—With few exceptions, the specimens studied were obtained from patients in the wards and clinics of Bellevue Hospital Center and the New York Veterans' Administration Hospital. In the case of the arthritic patients, the following specimens were obtained whenever possible in each instance: synovial fluid by aspiration; synovial membrane by open or needle biopsy; and conjunctival secretions with a sterile cotton swab. In females, vaginal secretions were taken with a sterile swab, and cervical and urethral scrapings with a sterile loop or swab. In males, urethral scrapings from a depth of 1.5 to 2 cm. were obtained as above after washing the meatus and glans, urine was collected before and after prostatic massage, and prostatic fluid was obtained by massage. In the case of patients without rheumatic diseases, the synovial fluid and synovial membrane specimens were, of course, omitted. Miscellaneous materials cultured included pus from a dog-bite wound, blister fluid from a patient with a cutaneous mycotic infection, stools, ascitic fluid, whole blood or serum, and pericardial and pulmonary tissues obtained at operation.

Virological Studies.—HeLa cells, monkey kidney, human synovial, and human amnion cells were employed for testing the pathological material for the presence of viruses. Chick embryos were not used because of the

possibility that they might already be infected by PPLO as reported by Klieneberger-Nobel (1960a, b). Chicken embryo extract, heparinized chicken serum, and Hank's balanced salt solution were prepared by usual methods and were tested for contaminating PPLO before use. Maintenance solution was prepared according to the method of Hayflick and Stinebring (1960). The growth media for the tissue cultures was prepared by adding four parts calf serum and one part chick embryo extract to five parts maintenance solution. For the human synovial cell cultures, the medium consisted of human ascitic fluid, chick embryo extract, and Gey's balanced salt solution. 100 units penicillin and 100 µg. streptomycin per ml. were added to both media.

For culturing the cell lines, the double-slide technique of Maximow (1929) was employed initially, but later the hanging cover-slip method of Hayflick and Stinebring (1960) was substituted. The human synovial cells were cultured on flying cover slips in Porter flasks. Giemsa staining was used according to the method described by Jacobson and Webb (1952).

Since the report of Robinson, Wichelhausen, and Roizman (1956) that a human cell line used by them was contaminated with PPLO, many of the generally employed tissue culture lines have been shown to harbour these organisms (Wittler, Cary, and Lindeberg, 1956; Collier, 1957; Chang, 1958; Rothblat and Morton, 1958, 1959; Nelson, 1960; Rothblat, 1960). Therefore, special care was taken to ascertain that the cultures used did not contain PPLO. Pieces of the tissues were inoculated into PPLO broth or were streaked out on a plate containing PPLO medium. The cultures were used only if three consecutive inoculations were negative. The monkey kidney and human amnion cells were prepared freshly for each lot of experiments in order to reduce the possibility of the presence of a dormant contaminant.

The cultures were incubated for 2 weeks and were examined for cytopathological effects. For the detection of inclusion bodies, Giemsa staining was employed as previously mentioned. If degeneration of the cell lines occurred before the ninth day, the tubes were discarded and new cultures were started. The pathological material received was kept at -20°C . if not used immediately.

Studies of Tissue Explants.—For the detection of virus changes in the explants of human tissues, cytopathological changes were sought as described above.

For the detection of PPLO in the synovial explant, pieces of tissues obtained by biopsy were cultured by the hanging cover-slip method as mentioned above. Fluids aspirated from the joints were added to preformed chicken plasma clots, prepared on individual cover slips according to Maximow's double-slide culture method, and were sealed in depression slides with paraffin.

PPLO Studies.—The pathological material was immediately inoculated on to PPLO plates at the location where it was obtained. Early in the investigation part of the prostatic fluid was collected directly in tubes filled

with Difco PPLO broth, but this method was subsequently discarded because of the high incidence of contamination; and henceforth only solid media were used. Media were prepared according to the method of Dienes (1939), Dienes and others (1948), Dienes, Weinberger, and Madoff (1950), Dienes and Weinberger (1951), and Madoff (1960). In each case the same pathological material was placed on each of two plates, one of which contained penicillin. Using the method of Fortner (1928), the cultures were made anaerobic by fixing a portion of a horse blood agar culture of *Serratia marcescens* on the cover of the Petri dish. After the dish was inoculated, it was sealed with paraffin. The cultures were examined routinely at 48 and 96 hrs. A specimen was called negative only if no PPLO could be demonstrated in three subsequent sub-cultures.

The identification of PPLO was based upon the generally-accepted morphological characteristics (Sabin, 1941; Dienes, 1945):

- (1) Presence of typical "fried egg" colonies;
- (2) Firm attachment of the colonies due to the growth of the centres into the media;
- (3) Colonial forms made up of two types of elements, small granules on the border of visibility and large bodies;
- (4) Penicillin resistance;
- (5) Need for special protein nutrients for growth.

The differentiation of penicillin-induced L-forms from PPLO did not cause special problems because of their bigger and much coarser structure (Dienes, 1942; Dienes and Weinberger, 1951; Klieneberger-Nobel, 1954, 1956, 1958, 1962; Freundt, 1958).

Special attention was given to recognition of the T-forms of Shepard (1954, 1956, 1957a, b, 1958, 1960). In the search for these forms, observation of the epithelial cells of synovial biopsy specimens on the agar surface provided a starting point. The synovial tissue pieces were cut into small fragments under sterile conditions and the plates were inoculated with these. For the investigation of the developed cultures, the direct agar block method of Ørskov (1922, 1927) was used. Small pieces of agar were cut from the media and placed on a slide with the inoculate exposed to view. Usually only such parts of the agar were cut as suggested the presence of PPLO after examination by a hand lens. The agar blocks then were covered with a cover slip, pre-stained with the "blue stain" of Dienes (1939), Dienes and others (1950), Madoff (1960). Paraffin was poured around the four uncovered sides of the block to prevent it from drying. Because of the possible presence of T-forms in our material, the whole surface of the block was examined with the aid of a $\times 93$ oil immersion objective and a $\times 8$ or $\times 25$ ocular.

From the plates containing the synovial tissue pieces, agar block preparations were made, as described previously, without disturbing the tissue fragments. In these preparations there were always detectable tissue areas

where the cells composed only a single or double layer. This made it possible to detect by direct microscopic examination any PPLO occurring in the tissue cells. Since part of the biopsy material was used for the preparation of routine histological slides, the opportunity was presented to examine fixed, haematoxylin-eosin stained slides* in cases in which the presence of PPLO was suspected in the agar block preparations.

Complement-Fixation Studies.—The procedure followed was that described by Kabat and Mayer (1948), as modified at the Pasteur Institute in Paris and conveyed to us by Miss Sarabelle Madoff and Dr Louis Dienes of the Massachusetts General Hospital in Boston. We are indebted to Dr Dienes also for the PPLO antigen which had been prepared by him for similar studies. Sheep erythrocytes stored in Alsever's solution, amboceptor, and guinea-pig complement originated from the commercial stock of the Carworth Farm Laboratories. In order to check our system, high titre PPLO antisera were prepared in rabbits by the subcutaneous administration of an oily suspension of the PPLO antigen into the foot pad and back.

Results

Virus Studies

The results of the virological studies are summarized in Table I (opposite), which shows that we were unable to demonstrate viruses in any of the tissues or fluids examined by the methods employed.

Explantation Studies

As shown in Table II, the explants from patients revealed no cytopathological changes which might have indicated the presence of virus or PPLO.

TABLE II
EXPLANTS TESTED FOR THE PRESENCE OF VIRUSES OR PPLO

Name of Disorder	No. of Patients	No. of Tissues	No. of Synovial Fluids	No. of Positive Tissue Cultures
Ankylosing Spondylitis	1	1	—	0
Gonococcal Arthritis	1	1	1	0
Psoriatic Arthritis	1	1	—	0
Reiter's Disease	4	4	2	0
Rheumatoid Arthritis	1	1	1	0
Tendovaginitis	1	1	—	0
Traumatic Joint	1	1	1	0
Total	10	10	5	0

FPLO Studies

The results of attempts to isolate PPLO are summarized in Table III (opposite).

*The authors wish to express their thanks to Dr Norman Cooper for providing the histological slides.

TABLE I
PATHOLOGICAL SPECIMENS TESTED FOR THE PRESENCE OF VIRUSES

Diagnosis	No. of Patients	Type of Specimens Cultured	No. of Specimens	Tissue Cultures Strains and Lines				Total No. of Cultures	No. of Positive Cultures
				HeLa	Monkey Kidney	Synovial Cells	Human Amnion		
Ankylosing Spondylitis	2	Prostatic fluid	1				1	1	0
		Synovial fluid	1				1	1	0
		Synovial tissue	1				1	1	0
Gout	2	Synovial fluid	1				1	1	0
		Synovial tissue	1				1	1	0
Psoriatic Arthritis	4	Prostatic fluid	1				1	1	0
		Synovial fluid	2	1	1	1	1	4	0
		Urine	4	2	2	2	2	8	0
Reiter's Disease	7	Synovial fluid	1				1	1	0
		Synovial tissue	4				2	8	0
		Urine	7	2	2	2	2	21	0
Rheumatoid Arthritis	6	Lung tissue	1				1	1	0
		Synovial fluid	2	1	1	1	1	4	0
		Synovial tissue	2	1	1	2		4	0
		Urine	2				2	2	0
Rheumatic Pericarditis	1	Pericardial effusion	1		1	1	1	3	0
		Pericardial tissue	1		1	1	1	3	0
Ulcerative Colitis with Arthritis	2	Stool filtrate	1				1	1	0
		Synovial fluid	1				1	1	0
		Urine	2				2	2	0
Total	24		37	14	16	17	22	69	0

TABLE III
DIAGNOSES OF PATIENTS AND TYPE OF PATHOLOGICAL SPECIMENS TESTED FOR THE PRESENCE OF PPLO

Diagnosis	No. of Patients	Type of Specimens	No. of Specimens and Cultures	No. of Positive Cultures	No. of Positive Cases	Per cent. of Positive Cases
Ankylosing Spondylitis	4	Prostatic fluid	4	0	1	
		Serum or blood	2	0		
		Synovial fluid	2	0		
		Synovial tissue	2	0		
		Tear fluid	2	0		
		Tooth scraping	1	1		
		Urine	6	0		
		Urine after prostatic massage	4	0		
		Urethral scraping	2	0		
		Total ..	25	1		
Chronic Cervicitis	4	Cervical scraping	3	0	2	
		Urethral scraping	3	1		
		Vaginal smear	5	3		
		Total ..	11	4		
Cirrhosis	6	Ascitic fluid	6	0	0	
Cystitis	9	Prostatic fluid	9	0	0	
		Urine	9	0		
		Urine after prostatic massage	9	0		
		Total ..	27	0		
Dermato-mycosis	1	Blister fluid	2	0	0	
Dog Bite	1	Pus	1	1	1	
Gout	4	Synovial fluid	4	0	0	
		Synovial tissue	4	0		
		Urine	7	0		
		Total ..	15	0		

Continued overleaf

TABLE III—continued

Diagnosis	No. of Patients	Type of Specimens	No. of Specimens and Cultures	No. of Positive Cultures	No. of Positive Cases	Per cent. of Positive Cases
Gonococcal Arthritis	1	Synovial fluid	1	0	0	
		Synovial tissue	1	0		
		Urine	2	0		
		Total ..	4	0		
Menopausal Bleeding	2	Cervical scraping	2	1	1	
		Urethral scraping	2	0		
		Vaginal smear	2	1		
		Total ..	6	2		
Vaginal Moniliasis	3	Cervical scraping	3	1	3	
		Urethral scraping	3	1		
		Vaginal smear	3	2		
		Total ..	9	4		
Non-gonococcal Urethritis	22	Prostatic fluid	17	1	5	
		Tear fluid	22	0		
		Tooth scraping	8	0		
		Urine	25	1		
		Urine after prostatic massage	16	1		
		Urethral scraping	15	3		
		Vaginal smear	4	2		
		Total ..	107	8		
						22.7
Pelvic Lesion	4	Cervical scraping	4	1	2	
		Urethral scraping	4	0		
		Vaginal smear	4	2		
		Total ..	12	3		
Post-gonococcal Urethral Stricture	16	Prostatic fluid	11	3	3	
		Tear fluid	32	0		
		Urine	16	3		
		Urine after prostatic massage	10	3		
		Urethral scraping	16	3		
		Total ..	85	12		
Postpartum Check-up	1	Cervical scraping	1	1	1	
		Urethral scraping	1	1		
		Vaginal smear	1	1		
		Total ..	3	3		
Pregnancy	2	Cervical scraping	2	2	2	
		Urethral scraping	2	1		
		Vaginal smear	2	2		
		Total ..	6	5		
Psoriatic Arthritis	5	Serum	2	0	0	
		Synovial fluid	3	0		
		Synovial tissue	2	0		
		Urine	6	0		
		Total ..	13	0		
Reiter's Disease	13	Prostatic fluid	12	0	3	
		Serum or blood	7	0		
		Synovial fluid	11	0		
		Synovial tissue	8	2		
		Tear fluid	24	1		
		Tooth scraping	12	0		
		Urine	26	0		
		Urine after prostatic massage	10	0		
		Urethral scraping	12	0		
		Total ..	122	3		
						23.0
Rheumatoid Arthritis	12	Lung tissue	1	0	0	
		Periosteum	1	0		
		Prostatic fluid	4	0		
		Serum or blood	4	0		
		Synovial fluid	8	0		
		Synovial tissue	6	0		
		Urine	5	0		
		Urethral scraping	6	0		
		Total ..	35	0		

Continued opposite

TABLE III—continued

Diagnosis	No. of Patients	Type of Specimens	No. of Specimens and Cultures	No. of Positive Cultures	No. of Positive Cases	Per cent. of Positive Cases
Tendo-vaginitis	1	Ganglia	2	0	0	
Traumatic Derangement of Joint	1	Synovial fluid	1	0	0	
		Synovial tissue	1	0		
		Total ..	2	0		
Ulcerative Colitis with Arthritis	2	Synovial fluid	1	0	0	
		Synovial tissue	1	0		
		Stool filtrate	1	0		
		Urine	3	0		
		Total ..	6	0		
Total	114		499	46	24	21.0

Taking the group of patients as a whole, it is apparent that PPLO were isolated relatively frequently from gynaecological and urological material from patients with genito-urinary disorders and especially from females, but rarely from other specimens. Thirteen (45 per cent.) of 29 female patients tested gave positive gynaecological cultures. In contrast, positive cultures were obtained in only twelve (14.1 per cent.) of 85 males, a surprisingly small number. A total of 499 specimens of all types were taken from 114 patients; 46 (9.2 per cent.) of these specimens from 25 (22 per cent.) of the patients were positive for PPLO.

In these studies the majority of the patients were included primarily as controls, and we were chiefly interested in the 39 patients with arthritis, and particularly in the thirteen with the diagnosis of Reiter's disease. As shown in Table III, all cultures from the twelve patients with rheumatoid arthritis and from the five with psoriatic arthritis were negative, and only a single culture from a tooth was positive in one of the four patients with ankylosing spondylitis. This strain was lost before it could be typed, but presumably it was a normal, oral, saprophytic form. All the thirteen synovial fluids and ten synovial tissues cultured in these patients gave negative results.

Among the thirteen patients with the diagnosis of Reiter's disease, all cultures of prostatic secretions and urine were negative. In one patient, PPLO were obtained from the apparently normal right eye while the severely inflamed left eye gave negative cultures. Since it is known that PPLO is a rare saprophyte in the conjunctival sac (Holland, 1960) and since these organisms could not be isolated from any other investigated area of this patient, it is improbable that the positive culture from the normal eye had aetiological significance.

Finally, although cultures of synovial fluid were negative for PPLO in all of the thirteen patients tested, cultures of synovial tissue specimens on PPLO plates were questionable in one and positive in another. Brief case reports of these two patients are given below.

Case 1, a male aged 28, had had his first attack of urethritis in 1953, 18 days after sexual intercourse. This was followed in a week by conjunctivitis lasting a few days, and 5 days later by arthritis of the left knee, and pain, tenderness, and swelling of the left Achilles tendon. The arthritis cleared completely in about 2 months. Apart from pain in the left knee in 1957, lasting for one week and not accompanied by any other complaints, he was then well until April, 1960, when urethritis again appeared 2 weeks after sexual intercourse. Conjunctivitis was not noted this time, but arthritis appeared 2 weeks after the start of urethritis. Bilateral Achilles tendonitis appeared first, and then both knees and ankles and the interphalangeal joint of the left thumb were involved in rapid succession. The thumb joint cleared in a few days, but the others continued to be affected for several months and the urethritis lasted for 5 weeks. The erythrocyte sedimentation rate was 41 mm. in one hour (Westergren). Latex-fixation and sheep erythrocyte agglutination and inhibition tests (Ziff, Brown, Lospalluto, Badin, and McEwen, 1956) were negative for rheumatoid factors. A synovial biopsy was performed on May 31. Fluid aspirated at that time was negative on culture for gonococci and other bacteria. The serum uric acid level was normal and an L.E. test was negative.

The tissue obtained by the needle biopsy was cultured on PPLO plates. It contained three cells which showed small rounded bodies similar to those described by Shepard (1954, 1956, 1957a, b, 1958, 1960) in urethral epithelial cells. These small granules were located to one side of the nucleus as small aggregates. The three cells contained 17, 9, and 5 such granules respectively, but manifested no

other pathological change. Since only three cells of many hundreds examined showed these granules and since they did not develop into T-type colonies, it cannot be more than suspected that they were PPLO.

Case 2, a male aged 23, had first observed urethral discharge on January 19, 1959. This was diagnosed as gonorrhoea and he received penicillin injections on three successive days. On January 26, he noticed pain in the knees and ankles which rapidly increased and led to his admission to Fort Ord Hospital on January 29. Within a few days, the right knee and both feet swelled. During the first 2 weeks in the hospital, the pain appeared also in the left elbow and right wrist with swelling of the former. Pain and stiffness also were noted in both hips. The erythrocyte sedimentation rate was rapid. On February 9 there was considerable pain in the right knee which became hot, red, and swollen. On February 13, 30 ml. "semi-purulent" fluid was aspirated, which on culture was positive for *Staphylococcus aureus* (coagulase positive). He was given tetracycline, and this was changed to chloramphenicol 500 mg. every 8 hours, starting on February 15. The swelling in the right knee rapidly subsided on this regimen plus rest in bed, skin traction, and ice packs to the affected joint. On February 16 and March 17, clear fluid which was negative on culture was aspirated from the right knee. Although the acute septic arthritis of the right knee subsided, the subacute arthritis continued in multiple joints. He was transferred to Letterman General Hospital on March 10, 1959, and on May 24, 1960, to the New York Veterans' Administration Hospital where we first saw him. There was no record of conjunctivitis in this case.

In the V.A. hospital, latex-fixation tests were negative as were several attempts to isolate micro-organisms from the synovial fluid of the right knee. That knee, as well as those in other cases previously noted, was still painful on motion, tender, swollen, and warm; but there was no erythema, the synovial fluid had a normal appearance, and the course of the disease was chronic and not at all suggestive of pyogenic arthritis, even in the right knee. On June 1, 1960, synovial tissue was obtained from the right knee by needle biopsy.

The synovial tissue obtained by needle biopsy on June 1, 1960, gave abundant growth of PPLO colonies. These were seen immediately beneath the tissue inoculum, while in the furrows caused by the streaking of the material some scattered colonies of *Staphylococcus aureus* were observed. The fact that this joint had previously been the site of staphylococcal infection and that some viable staphylococci were still apparently present in the synovial tissues, immediately suggests that the PPLO isolated from the same tissue specimen were L-forms derived from the staphylococci. Against this view, however, are the following points: as previously

mentioned, two plates were used for the primary inoculation: one with and one without penicillin. Both the PPLO and the staphylococci grew on each plate. The staphylococci showed only slight pleomorphism on the plate containing penicillin, suggesting that this was a resistant strain. No growth forms of the staphylococcus were seen which resembled L-forms that might be mistaken for PPLO. Furthermore, the isolated PPLO strain was sub-cultural several times in penicillin-free media and was maintained for more than a year without showing any tendency to revert to a bacterial form. Moreover, Dr Louis Dienes, who kindly examined this micro-organism for us, considered it to be a true PPLO.

The PPLO colonies in the primary inoculum closely resembled the T-type described by Shepard or the "very small" genital strain of Dienes and Madoff (1953) (Figs 1 and 2, opposite). The colonies from our patient possessed some features, however, which differed from Shepard's T-type colonies. They did not show such a clearly-defined tendency to grow into the media. Furthermore, the shape of the primary colonies was more amorphous than circular, and their diameter rarely exceeded 10μ (Fig. 3, opposite).

In the cells of the synovial membrane there were a great many small granules similar to those described for Case 1; in two cells, forms resembling "large bodies" were seen (Fig. 2). Shepard, however, did not mention the occurrence of large bodies in the tissues. These large bodies were ovoid in shape with a longitudinal diameter of about 2.5μ and a width of 1.5 to 2μ . They presented a well-stained peripheral zone with very slight granularity and a nearly colourless homogeneous central area. Study of haematoxylin and eosin-stained tissue sections revealed small, faintly-staining granules of the same size and located in the same place as those in the agar-block preparations. In subcultures, these organisms never grew into typical "fried egg" colonies, but showed a surface growth with small granules aggregating in amorphous clumps and large bodies around them (although certain parts of the colony did grow actively into the media) (Fig. 4, opposite).

When a heavy inoculum was used, the growth form had a great resemblance to the 4330 strain of Dienes originally cultured from female genitalia, but our colonies were smaller (Fig. 5, opposite). The organism met all the criteria required for identification as PPLO: it was penicillin resistant, the small granules were at the border of visibility, the colonies were composed of two different elements (granules and large bodies), and part of the colony (although not always

FIG. 1.—Small PPLO colony developing below a synovial cell. Primary inoculum 48 hrs. Note outlines of synovial cells.

FIG. 2.—Same as Fig. 1, focused for the few large bodies.

FIG. 3.—Same as Fig. 1, but 72 hrs.

FIG. 4.—Small PPLO colony after first transfer, 96 hrs.

FIG. 5.—PPLO colonies after second transfer, 36 hrs.

FIG. 6.—4330 human genital strain which was kept under unfavourable conditions (prolonged time lapse between transfers), included for sake of comparison. Note similarity to Fig. 4.

All pictures taken with a Leitz phase-contrast microscope, using a $\times 93$ oil immersion objective and a $\times 10$ ocular.

the central part) grew into the agar. We did not attempt to define the biochemical characteristics other than to observe that these micro-organisms were capable of decolourizing methylene blue within 45 to 60 minutes. Older colonies did not stain with the "blue stain", a feature observed by Shepard in his T-types. As in the previous case (Case 1), we could not demonstrate the presence of PPLO in the prostatic secretions, the urine, or the eyes, and even the synovial fluid, taken concomitantly with the tissue, was found to be sterile. The patient's serum was also negative for PPLO antibodies in complement fixation tests.

In agreement with Nasemann and Röckl (1960), we were unable to find any relationship between the occurrence of PPLO and *Trichomonas vaginalis*, as was suggested by Peoples, Morton, and Feo (1957). From the 29 female patients investigated, twelve had trichomoniasis, while thirteen proved to harbour PPLO. In six cases, PPLO and *Trichomonas* occurred together in the same person, in seven we found PPLO without *Trichomonas*, and in six *Trichomonas* without PPLO.

Some correlation can perhaps be inferred between the occurrence of vaginal moniliasis and the presence of PPLO, although our cases are too few to have statistical significance; for PPLO were found in all cases in which moniliasis was present. Indeed, it became routine to search for PPLO colonies around the hyphae of the fungus, which could be easily found by examination under low power magnification.

In spite of the most painstaking effort, we could not confirm the 70 per cent. occurrence of T-type PPLO reported by Shepard (1960) in cases of non-gonococcal urethritis. As shown in Table III, we could identify PPLO in only 22.7 per cent. of our patients with that diagnosis, which is within the limits of their occurrence in apparently normal subjects. The discrepancy between Shepard's results and ours may be due to differences in the populations investigated, since it is well known that the number of positive PPLO cultures increases with the degree of promiscuity in a given population group (Shepard, 1954; Klieneberger-Nobel, 1960).

No correlation was found between the occurrence of PPLO in our patients and their proximity to rodents or household pets, as was suggested by Dienes and others (1948), Nelson (1958), and Kuzell and Mankle (1960).

Complement-Fixation Studies

The results are summarized in Table IV.

As is apparent, only one person with Reiter's disease gave a titre as high as 512. Of nine patients

TABLE IV
COMPLEMENT-FIXATION TITRES OF ANTISERA TESTED
IN ELEVEN CASES OF REITER'S DISEASE

Patients	Complement-Fixation Titre of Patient Antisera v. Campo Antigen	Complement-Fixation Titre of Rabbit Antiserum v. Campo Antigen (Control)
S.R. . . .	8	2048
G.M. A9739 . . . (Case 1 in text)	64 32	2048 2048
F.B. 213160* . .	128	2048
X.Y.† . . .	32	2048
N.R.E. . . .	512	2048
J.O. A6016 . . . (Case 2 in text)	64 32	2048 2048
S.W. A9540 . .	16	2048
L.S. 12911-60 . .	64	2048
A.S. . . .	8	1024
C.F. . . .	16	1024
L.J. 2064-56‡ . .	16	1024

* Serum received from Presbyterian Hospital.

† Serum received from Boston Veterans Administration Hospital.

‡ Joint fluid used instead of serum.

investigated, five had titres of 64 or higher, but two of these patients had even lower antibody levels when the tests were repeated 2 and 5 weeks later. It is known (Card, 1959; Klieneberger-Nobel, 1960) that a significant proportion of the population has circulating PPLO antibodies and the low titres obtained in our patients cannot be interpreted as of pathogenetic significance. Furthermore, the titres were normal in the one patient in whom PPLO were unquestionably present in the synovial tissues. These findings and those of others (Weinberger and Bauer, 1955; Melén and Gotthardson, 1955; Willcox, 1958; Csonka, 1959; and Oates, Whittington, and Wilkinson, 1959) show that complement-fixation techniques can shed little if any light on the question of involvement of PPLO as the cause of Reiter's disease or of non-gonococcal urethritis, as was claimed earlier by Beveridge and others (1946).

Discussion

As was noted at the start of this paper, there are three principal theories as to the cause of Reiter's disease:

- (1) An unidentified virus.
- (2) Pleuro-pneumonia-like organisms.
- (3) Hypersensitivity of delayed type to gonococci and perhaps also to *Shigellae*.

The studies here reported were undertaken to explore the first two of these theories, but have added little to what was already known. No evidence of virus infection was found either by direct isolation or by cytotoxic changes in tissue cultures. The attempts to isolate PPLO tended to confirm previous reports of the presence of these micro-organisms in the urogenital tracts of normal females and males and of those with various gynaecological and urological disorders. Results were negative in the patients with Reiter's disease with the exception of one, and possibly two, of the studies made on biopsy specimens of synovial tissues. The literature contains references to two positive cultures of synovial fluids (Dienes and others, 1948). To our knowledge, tissue specimens of synovial membranes have not previously been used for cultures and, whereas the meagre results in our few patients certainly do not justify drawing conclusions as to the significance of PPLO in Reiter's disease, we believe them to warrant additional effort to isolate these micro-organisms from synovial tissues. Such studies are currently in progress as suitable patients present themselves. Since patients with Reiter's disease are not frequently seen in our clinics, we hope that other workers will also make use of synovial membrane specimens for cultures.

The one patient in this series with a definitely positive synovial membrane culture for PPLO had previously had a staphylococcal infection of the biopsied joint and staphylococci also grew in small numbers on the PPLO medium, although not from cultures of the synovial fluid. At the time of biopsy, the joint did not present clinical features of pyogenic arthritis and the other involved joints in this patient were at no time thought to be the seat of staphylococcal arthritis. Whether the presence of residual staphylococci made isolation of the PPLO easier cannot be said. Dienes and others (1948) also noted staphylococci in their cases of positive PPLO cultures from joint fluid. The isolation of PPLO from a joint containing cocci naturally suggested that the latter might be staphylococcal L-forms. This, however, is considered improbable because the PPLO and the staphylococci were found on plates with and without penicillin, and this patient had received no penicillin for at least eight months prior to the biopsy. The staphylococci showed only slight pleomorphism on the penicillin plate, suggesting that the strain probably was resistant to penicillin. Furthermore, the PPLO isolated showed the morphological features which are thought to distinguish genuine PPLO from L-forms of bacteria (Dienes, 1939, 1942, 1945, 1960; Dienes and others, 1950; Dienes and Weinberger, 1951; Dienes and Madoff,

1953; Liebermeister, 1953; Kandler and Kandler, 1954; Klieneberger-Nobel, 1954, 1956, 1958; Madoff, 1960).

Summary

Attempts were made to demonstrate viruses and PPLO in body fluids and synovial tissues of patients with various rheumatic diseases. All attempts to demonstrate viruses were negative. PPLO were looked for in 499 specimens from 114 patients with rheumatic and non-rheumatic diseases. Whereas these organisms were readily isolated from genito-urinary specimens, they were found in none of 31 synovial fluid specimens. They could not be grown from synovial tissue biopsies of patients with rheumatoid arthritis, ankylosing spondylitis, gout, gonococcal arthritis, psoriatic arthritis, traumatic arthritis, or arthritis accompanying ulcerative colitis, but they were present in one and possibly in two of eight synovial tissue specimens from patients with Reiter's disease. Complement-fixation tests were negative in nine patients with Reiter's disease, including the two from whose synovial tissues PPLO were thought to have been isolated.

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Études microbiologiques du syndrome de Reiter

RÉSUMÉ

On essaya de démontrer les virus et les organismes semblables au microbe de la péripneumonie des bovidés (PPLO) dans les humeurs et dans le tissu synovial de malades atteints de maladies rhumatismales de tout genre. Aucun virus ne fut découvert. On chercha les PPLO dans 499 spécimens de 114 cas de maladies rhumatismales et non-rhumatismales. Les organismes furent trouvés sans difficulté dans les souches génito-urinaires mais pas du tout dans 31 spécimens de synovie.

On ne trouva pas de PPLO dans les tissus synoviaux des malades atteints de polyarthrite chronique évolutive, de spondylite ankylosante, de goutte, d'arthrite gonococcique, psoriasique ou traumatique, ou d'arthrite associée avec la colite ulcéreuse, mais les organismes furent présents dans un (et peut-être dans deux) des 8 spécimens de tissu synovial prélevés sur des malades atteints du syndrome de Reiter. Les tests fixant le complément furent négatifs dans 9 cas de maladie de Reiter, y compris les deux des tissus synoviaux de qui on pensait avoir isolé des PPLO.