T-STRAIN PLEUROPNEUMONIA-LIKE ORGANISMS AS ONE CAUSE OF NON-GONOCOCCAL URETHRITIS*

BY

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A recent study and review of the literature (Ford, 1960) provided no support for the view that the usual strains of genital pleuropneumonia-like organisms (PPLO) were a cause of non-gonococcal urethritis (NGU). It was noted, however, that Shepard (1956, 1957, 1960) had isolated morphologically distinct T-strain PPLO from the genital tract of patients with NGU, and that he believed these T-strain PPLO to be aetiologically related to the urethritis. The present work was undertaken to seek confirmation of Shepard's findings in a different geographical area.

Materials and Methods

The cultural and staining techniques of Shepard were employed and precisely followed; moreover, for setting up the procedures, Dr. Shepard very kindly provided samples of his media. The studies were performed on patients attending the Vancouver Venereal Disease Control Clinic of the British Columbia Department of Health, and the great majority of the patients with NGU had a purulent or sero-purulent discharge of less than 2 weeks' duration. Some had been treated with penicillin or sulphonamides but none had received streptomycin or broad-spectrum antibiotics. Urethral scrapings were obtained with a platinum loop introduced about 2 inches into the urethra, and, when such deep urethral specimens were obtained, the bacterial contamination was less than when a meatal scraping was employed. Most of the patients had urethral cultures taken on two occasions at 3-day intervals, and smears for staining with Giemsa were prepared at the time the scrapings were taken.

Two types of male "controls" were used for comparison with the cases of NGU:

- (i) 25 inmates of the city jail, who volunteered, were studied by a deep urethral scraping after prostatic massage, the latter procedure being used because the scraping of a dry urethra yielded insufficient material for study.

- (ii) Thirty male patients without recent genito-urinary disease were investigated in the Vancouver General Hospital by meatal swabbing.

Results

T-strain PPLO colonies with the morphological characteristics described by Shepard were found and recognized by their small size in comparison to the more usual large PPLO forms. They could also be differentiated from the large colonies of the usual strains of PPLO by their slightly paler sky-blue colour and somewhat more refractile granules. There seemed to be no indication that the T-strains developed into larger colonies with further incubation, and in mixed cultures there was rarely any difficulty in distinguishing the two types.

Fig. 1 (opposite) illustrates the differences in colony size and refractility of the granules. Shepard describes subcellular ramifying (TR) and subcellular cluster (T-C) colonies, and it was thought that these forms were seen on several occasions and differentiated from cellular debris and pseudopodia by their typical PPLO sky-blue colour.

The possibility that penicillin-induced L-phase bacterial colonies could confuse the findings of the present study does not apply to the results in the group of NGU cases, because in only two of the 27 cases did the diagnosis of T-colonies depend on penicillin plates. Moreover, over half the T-strain PPLO isolations were obtained in cases of abacterial urethritis. In the groups of gonococcal and control cases, however, it was necessary to rely on penicillin agar because of the number of contaminating bacteria; and the extent of bacterial contamination in the "prostatic massage scrapings" of the controls was remarkable. On rare occasions in penicillin

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FIG. 1.—“Large colony” and T-strain PPLO colonies in an agar block preparation stained by Dienes' method and photographed with an oil-immersion objective.

plates there was difficulty in distinguishing T-strain colonies from small L-phase bacterial colonies and it is possible, therefore, that the recorded incidence of T-strains in the gonococcal and control cases might be higher than the true incidence. Further studies of the morphology of early L-phase bacterial colonies and their differentiation from T-strain PPLO colonies are under way.

Table I gives the cultural findings in the various categories of patients studied, and the data for the T-strain and “large colony” PPLO are recorded separately. T-strain PPLO were isolated from each

group and the incidence in the patients with gonorrhoea was similar to that in the controls, 26 and 22 per cent respectively. The incidence in the NGU cases was 60 per cent., and the χ^2 test was applied to the figures for the isolation of T-strain PPLO from the cases of NGU as compared with the “prostatic massage” controls who had a higher incidence than the “meatal swab” controls. The value for P which was derived from this statistical calculation was slightly more than 0.05, indicating that these findings could have occurred by chance once in about nineteen observations.

TABLE I

INCIDENCE OF T-STRAIN AND “LARGE COLONY” PPLO IN CONTROLS, PATIENTS WITH GONORRHOEA, AND PATIENTS WITH NON-GONOCOCCAL URETHRITIS

Classification of Cases			Controls		Cases of Gonorrhoea	Cases of Non-gonococcal Urethritis		
			Meatal Swab	Prostatic Massage		Total No.	Abacterial Urethritis	Bacteria Present
No. of Cases			30	25	27	45	25	20
PPLO	T-strain . . .	No. . .	5	7	7	27	16	11
		Per cent.	17	28	26	60	64	55
	“Large Colony”	No. . .	7	2	6	8	4	4
		Per cent.	23	8	22	18	16	20

With the exception of the lower figure in the "prostatic massage" controls, the incidence of "large colony" PPLO was fairly uniform throughout the different clinical categories, varying between 16 and 23 per cent. These data resembled those of the previous study (Ford, 1960), in which the incidence of "large colony" PPLO in patients with NGU was found to be no higher than in controls or in patients with gonorrhoea. The findings, in this study, of an 18 per cent. incidence of "large colony" PPLO in the 45 patients with NGU, is remarkably similar to the incidence of 21 per cent. in the 63 patients previously investigated.

Giemsa-stained slides of the urethral scrapings were available for study in 39 of the 45 NGU cases. Characteristic inclusions, as described by Shepard and as illustrated in Fig. 2, were found in fourteen of 23 cases which yielded T-strain PPLO on culture, and only five of the 23 cases yielding T-strains failed to show evidence of definite or probably PPLO in the smears. In contrast, inclusions were found in only one of fifteen cases in which T-strain colonies were not cultured. The demonstration of inclusions was not easy and cells having inclusions were often sparse in the scrapings, so that about 500 epithelial cells had to be studied in each case before an opinion could be expressed on the presence or absence of inclusions. As shown in Table II, one case revealed extra-cellular bodies that were considered to be PPLO granules.

TABLE II
INCIDENCE OF PPLO INCLUSIONS IN GIEMSA-STAINED
SCRAPINGS FROM 39 PATIENTS WITH NON-GONOCOCCAL
URETHRITIS

T-strain Colonies Cultured on Agar	No. of Cases	Inclusions			Probable Extracellular PPLO
		None	Definite	Prob- able	
Absent ..	16	15	1	—	—
Present ..	23	5	14	3	1

Discussion

The results of the present investigation confirm most of the findings of Shepard. Morphologically distinct colonial forms of PPLO were found having the characteristics of T-strain PPLO as described by Shepard. The presence of these T-strain organisms on culture was associated with epithelial cell inclusions. T-strain PPLO were found in 60 per cent. of cases of NGU and, most important, sixteen of 25 cases of abacterial urethritis yielded these organisms. Nevertheless, the significance of T-strain PPLO in the aetiology of NGU is hard to evaluate from the present data alone, because T-strain PPLO were found in an appreciable proportion of controls. Incidence data do not, however, provide the only available information, and the response of patients to antibiotics can be used as circumstantial evidence to support Shepard's contention. Most cases of abacterial NGU do respond rapidly to both streptomycin and the broad-spectrum antibiotics, which

FIG. 2.—Cytoplasmic inclusions of T-strain PPLO in an urethral epithelial cell stained with Giemsa and photographed with an oil-immersion objective.

suggests that the aetiological agent may be a sensitive organism such as a PPLO. Moreover, Shepard (1961) has correlated the disappearance of organisms with the remission of symptoms under antibiotic therapy, and has observed simultaneous recurrence of symptoms and organisms in patients having relapses following inadequate antibiotic therapy.

It has been suggested that T-strain PPLO are artefacts arising in particular cultural conditions, and inevitably, because they are PPLO, it is quite possible that other cultural conditions might give different colonial characteristics. It remains true, however, that under the conditions described by Shepard, two morphologically distinct forms do exist, and the different forms do appear to have separate relationships to NGU. The observation of Klieneberger-Nobel (1959) that 48 per cent. of 65 cases of acute or subacute NGU revealed "large colony" PPLO remains a contrast to the work described here in which the incidence of "large colony" PPLO was about 20 per cent. Shepard (1961), whose techniques we were following, also reported a low incidence of large colony PPLO in his cases of NGU; however, he has observed that Klieneberger-Nobel's medium favours the growth of large colony PPLO as compared to his medium.

The response of patients with abacterial urethritis to tetracycline suggests that over 90 per cent. of cases are caused by PPLO. However, in the present data and that of Shepard, T-strain PPLO have been found in only 60 to 70 per cent. of cases, so that some which respond to tetracycline have not yet revealed T-strains on culture. These cases present a challenge, as does the fact that T-strain colonies cannot be subcultured, and both observations demand an improvement in the present techniques for isolating and growing T-strain PPLO.

The relationship of Reiter's syndrome to the T-strain PPLO is not clear and our experience is too small to express an opinion. However, the arthritis and other features of the syndrome appear to be resistant to tetracycline therapy, which suggests that, if T-strains are related to the syndrome, the relationship may be an indirect one, possibly through a hypersensitivity mechanism. On the other hand, Shepard has observed some T-strains which have been resistant to a daily dose of 2 g. tetracycline for 10 days; moreover, antibiotic-resistant PPLO have been described in diseases of chickens and in tissue culture systems. The arthritis and other symptoms complicating NGU and gonorrhoea may be due to a superimposed viral infection, but repeated attempts to reveal a virus in the urethral exudate of patients with NGU have been uniformly unsuccessful.

Recently, in our laboratory, additional tissue culture experiments have failed to reveal a cytopathogenic change in monkey kidney cultures inoculated with urethral exudate; haemagglutination and haemadsorption phenomena were not found in the inoculated cultures, and interference with 100 M.I.D. of ECHO-9 virus was not demonstrable.

Summary

45 patients with acute non-gonococcal urethritis (NGU), 27 patients with gonorrhoea, and 55 control individuals without urethritis were investigated by the techniques of Shepard for the study of T-strain PPLO. T-strain PPLO with the morphological characteristics described by Shepard were found in all categories of individuals, but the incidence of 60 per cent. in patients with NGU was over twice as great as that in any other group. Epithelial cell inclusions, as described by Shepard, correlated closely with the presence of T-strain PPLO on culture.

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Organismes microbiens ayant la morphologie générale du microbe de la peripneumonie des bovidés (P.P.L.O.) de souche "T": une cause de l'urétrite non-gonococcique.

RÉSUMÉ

On a examiné 45 malades atteints d'urétrite non-gonococcique aiguë et 55 témoins sans urétrite par la méthode de Shepard d'étudier les souches P.P.L.O.

On trouva des organismes qui montraient les caractéristiques morphologiques décrits par Shepard dans des individus de chaque catégorie, mais la fréquence de 60% chez les malades était deux fois plus grande que la fréquence chez les témoins. Les inclusions de cellules épithéliales, décrites par Shepard, s'accordèrent bien avec la présence des souches "T" P.P.L.O. en culture.