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INCIDENCE OF PLEURO-PNEUMONIA-LIKE ORGANISMS (P.P.L.O.) IN MAN*

BY

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Strains of P.P.L.O. were cultured from 1,121 persons, and the skin reactions to intradermal injection of P.P.L.O. suspension were observed in ninety persons.

Material and Methods

P.P.L.O. cultures were grown on a soft nutrient agar (0·8 per cent.) with horse serum (15 per cent.), and substances inhibiting the growth of undesired bacteria (thallium acetate 1 : 2000 (w/v) and penicillin 50 I.U./ml.) (Edward, 1947). Identification was by observation of the morphological characteristics of colonies and growth specificities, and the microscopic picture was studied with a phase-contrast microscope.

The material investigated in female cases consisted mainly of vaginal discharge, as positive results had been found more often in P.P.L.O. cultures from the vagina than from urethra or cervix. *Trichomonas vaginalis* was detected by Wright's staining method and in wet specimens (in one drop of 0·85 per cent. NaCl). The approximate count of neutrophils in the microscopic field was also determined.

* Abridged from a paper read at the European Symposium of the I.U.V.D.T. at Cracow, Poland, in September, 1960.

Results

Females.—The 504 women investigated all had an increased amount of vaginal discharge. A close relation between syphilitic and gonorrhoeal infections and the presence in the genito-urinary tract of P.P.L.O. strains (96 per cent.) was observed. P.P.L.O. and *T. vaginalis* occurred together in 78·6 per cent., and P.P.L.O. were associated with other inflammatory conditions of the female genito-urinary tract in 35·7 per cent.

The difference between the percentage of positive P.P.L.O. cultures in trichomoniasis and other inflammatory processes of the female genito-urinary tract proved to be statistically significant ($t = 10{\cdot}645$; $n > 120$; $\alpha < 0{\cdot}01$; $P > 99$ per cent.) (Table I).

Statistical analysis of the group of women infected with *T. vaginalis* showed no correlation between the severity of the inflammatory process and the positive results of P.P.L.O. cultures (Table II, overleaf), whereas this correlation was clearly apparent in the group without *T. vaginalis*. The assays carried out *in vitro* with *T. vaginalis* cultures and P.P.L.O. did not

TABLE I

OCCURRENCE OF P.P.L.O. IN THE VAGINA

Female Subjects		No. of Cases	P.P.L.O. Positive	
			No.	Percent.
Adults	Infected with *T. vaginalis*	257*	202	78·6
	Discharge free from *T. vaginalis*	235*	84	35·7
	With Primary and Secondary Syphilis	12 } 26	11 } 25	96·0
	*With Gonorrhoea	4	14	
	Total	504	297	58·9
Girls aged 3–10 yrs with Vulvovaginitis	Gonorrhoeal	3	2	
	Non-gonorrhoeal	12	3	
	Total	15	5	
Grand Total		519	302	58·2

TABLE II

STATISTICAL ANALYSIS OF CORRELATIONS BETWEEN SEVERITY OF INFLAMMATORY PROCESS AND POSITIVE P.P.L.O. CULTURES

Female Subjects	Average count of leucocytes in one microscopic field	No. of Cases	P.P.L.O.	
			Positive	Negative
*Infected with *T. vaginalis*	0–4	4	2	2
	4–10	39	33	6
	10–20	54	42	12
	20–40	98	77	21
	More than 40	62	48	14
	Total	257	202	55
†Discharge free from *T. vaginalis*	0–4	49	9	40
	4–10	71	21	50
	10–20	55	21	34
	20–40	44	25	19
	More than 40	16	8	8
	Total	235	84	151

* χ^2 = 3,191 (for $n=4$; $\alpha > 0{\cdot}5$; $P<50$ per cent.). This points to lack of correlation between severity of inflammatory process and incidence of P.P.L.O. in patients infected with *T. vaginalis*.

† χ^2 = 17,362 (for $n=4$; $\alpha < 0{\cdot}01$; $P>$ 99 per cent. This points to statistical correlation between severity of inflammatory process and incidence of P.P.L.O. in patients not infected with *T. vaginalis*.

help to explain this peculiar connexion between trichomoniasis and P.P.L.O. infections.

Five cultures were obtained from fifteen girls aged 3 to 10 years and from two virgins with vaginitis. This suggests that P.P.L.O. infection can arise non-sexually.

Males.—Urethral material was investigated from 237 males, including eighty healthy persons with no discharge, 44 with non-gonococcal urethritis (N.G.U.), 78 with venereal disease, and the remainder with other genital infections. Positive P.P.L.O. cultures were obtained in 102 cases (Table III).

The difference between the percentage of positive P.P.L.O. cultures in the N.G.U. group (43 per cent.) and in the controls (14 per cent.) was statistically significant ($t=3{\cdot}448$; $n>40$; $\alpha<0{\cdot}01$; $P>99$ per cent.), but there was no statistical difference between the occurrence of P.P.L.O. in cases of N.G.U. and in other inflammatory conditions of the male genito-urinary tract (excluding syphilis), so that the aetiological role of P.P.L.O. in N.G.U. seems to be doubtful ($t=0{\cdot}735$; $n>40$; $\alpha>0{\cdot}40$; $P<60$ per cent.).

P.P.L.O. were isolated from twenty of 27 syphilitic men investigated. Fourteen of these still showed primary syphilitic lesions on the penis from which P.P.L.O. could be cultured in seven cases. A close correlation of P.P.L.O. infection with syphilis could be due to simultaneous transmission, especially from prostitutes, who often harbour P.P.L.O. in the lower genital tract or to the fact that syphilitic primary lesions create exceptionally favourable breeding ground for P.P.L.O. Some other relationship between syphilis and P.P.L.O., perhaps analogous to that already mentioned between *T. vaginalis* and P.P.L.O. should not be excluded.

Special attention is drawn to the incidence of P.P.L.O. in semen, 51 swabs from the urethra and the semen of males reporting for consultation because of infertility were tested, and 26 positive cultures (51 per cent.) were obtained (Table IV). The incidence of P.P.L.O. strains in semen was not related to the normality of the semen.

TABLE III

OCCURRENCE OF P.P.L.O. IN THE MALE GENITO-URINARY TRACT

Diagnosis		No. of Cases	P.P.L.O. Positive	
			No.	Percent.
Non-gonococcal Urethritis		44	19	43
Gonorrhoea		26	8	
Chronic Urethritis after Gonorrhoea		22	5	
Primary and Secondary Syphilis ...		27	20	
Miscellaneous Inflammatory Conditions	Phimosis	5	3	
	Balanitis erosiva ...	7	5	
	Diphtheria penis ...	2	2	
	Epididymitis (gonococcal) ...	3	0	
	Ulcus phagedenicum penis	1	1	
		18	11	
* Infertility but no Pathological Urethral Discharge		51	28	55
Total Pathological Subjects		188	91	48·4
Healthy Controls		80	11	14
Grand Total...		268	102	

* 20 cultures from urethra and semen. 31 cultures from semen only.

TABLE IV

POSITIVE P.P.L.O. CULTURES FROM SEMEN

Semen	No. of Specimens	P.P.L.O.	
		Positive	Negative
Normal	35	19	16
Pathological	16	7	9
Total	51	26 (51 Per cent.)	25

PRESENCE OF P.P.L.O. IN URETHRA AND IN SEMEN

P.P.L.O.		Semen		
Urethra	Semen	Total	Pathological	Normal
+	+	10	4	6
+	—	2	1	1
—	+	0	0	0
—	—	8	5	3

P.P.L.O. often appears simultaneously in semen and urethra in cases with no discharge. It seems

probable that semen, owing to its high pH, presents especially favourable conditions for P.P.L.O. and that the male seminal tract may act as a natural reservoir for these germs. The role of P.P.L.O. in semen as a factor in human infertility is still uncertain. It should be borne in mind that the infertility of a couple is mainly dependent on previous pelvic inflammatory conditions in the female partner which are not infrequently accompanied by P.P.L.O. It is possible that the men investigated had been secondarily infected by their female partners. With one exception (Morton, Smith, and Keller, 1952), all other investigators have failed to find P.P.L.O. in human semen.

This problem is of some importance, because in animals (dogs and bulls) a causative relation has been found between the incidence of P.P.L.O. in male semen and infertility and inflammatory processes in the female genital tract (Edward, 1950; Edward and Fitzgerald, 1951).

Application of the χ^2 criterion in the evaluation of 707 cultures showed a significant increase in the frequency of positive results in the summer months ($\chi^2=12{\cdot}506$; $n=1$; $\alpha<0{\cdot}01$; $P>99$ per cent.).

No statistically significant difference was found between P.P.L.O. cultures on media with 1 : 500 (w/v) thallium acetate and those with 1:2,000 (w/v) with the addition of 50 I.U. penicillin per ml. ($\chi^2=3{\cdot}1099$; $n=1$; $\alpha>0{\cdot}05$; $P<95$ per cent.).

Intradermal Tests

The pathogenicity of P.P.L.O. may be evaluated from the skin reaction to the intracutaneous injection of P.P.L.O. antigens. For this purpose an "antigen" made from twenty various P.P.L.O. genital strains in Evans' fluid (Evans, 1922) in doses of 0·1 ml. were injected intracutaneously into the forearm. A 2 per cent. solution of the liquid culture medium was used as a control. The results were read after 24 and 48 hours. The appearance of a papule (at least 3 × 3 mm.) on an erythematous background was considered as positive. The reactions of ninety males and females were investigated, and 54 (60 per cent.) were found to be positive, sixteen (18 per cent.) being strongly positive. It was found that within 48 hours some of the reactions were strengthened whereas others became weaker. No relation between the presence of P.P.L.O. strains in the genito-urinary tract and the strengthening, weakening, or appearance of the skin reaction was noted (Table V). It is difficult to say whether the observed intradermal reaction is strictly specific in character or whether it is the result of allergy. However, the results show that a differentiated reactivity towards P.P.L.O. strains exists in humans, and this puts the question of the pathogenicity of this micro-organism on a new footing.

TABLE V

INTRADERMAL SENSITIVITY TESTING WITH A SUSPENSION OF P.P.L.O. (READ AFTER 48 HRS.)

Result of Sensitivity Test	No. of Cases	P.P.L.O. in Genito-urinary Tract	
		Positive	Negative
Positive	54	26	28
Negative	24	16	8
Uncertain	12	5	7
Total	90	47	43

Positive P.P.L.O. cultures were obtained from the oral cavity and pharynx in 196 of 334 males and females tested (seventeen were "genital" and 179 "oral" in type). In ninety cases, no connexion was found between the presence of P.P.L.O. in the genito-urinary tract and in the oral cavity (Table VI).

TABLE VI

ISOLATION OF P.P.L.O. FROM ORAL CAVITY

Subjects	Total No. Examined	P.P.L.O.	
		"Genital" Type	"Oral" Type
Healthy Medical Students	263	12	146
Hospitalized Patients ...	71	5	33
Total	334	17 (4·9 per cent.)	179 (53·8 per cent.)

P.P.L.O. IN GENITO-URINARY TRACT AND ORAL CAVITY IN SEVENTY PATIENTS ATTENDING THE DEPARTMENT OF DERMATOLOGY

P.P.L.O.		No. of Patients		
Genito-urinary Tract	Oral Cavity	Male	Female	Total
+	+	11	7	18
+	—	6	9	15
—	+	12	8	20
—	—	13	4	17
Total		42	28	70

The microbiological character of some strains was studied, using serial photomicrographs, and continuous observations in the Orskov-Peshkov camera with the aid of the phase-contrast microscope. The role of filament elements in P.P.L.O. development on solid media has been firmly established, and the initial development process and metamorphosis of the filaments in environmental conditions excluding

artefacts have been illustrated photographically. Staining with strongly diluted basic fuchsin and methylene blue in phosphate buffer (pH about 5), resulted in colour differentiation between P.P.L.O. colonies and epithelial scrapes in impression preparations. Further details concerning strictly microbiological investigations will be the subject of a separate publication.

Summary

The incidence of P.P.L.O. strains cultured from the genito-urinary tract and oral cavity in 1,121 persons is described.

The skin reactions following intradermal injection of a P.P.L.O. suspension were observed in ninety persons.

Some microbiological details characteristic of the micro-organism are discussed.

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L'incidence chez l'homme des organismes microbiens ayant la morphologie générale du microbe de la péripneumonie des bovidés (P.P.L.O.)

Résumé

L'incidence des souches P.P.L.O. prélevées dans les voies génito-urinaires et dans la cavité buccale de 1.121 personnes est décrite. Des cuti-réactions après injection intradermale de P.P.L.O.s en suspension furent observées chez 90 personnes. Certaines caractéristiques microbiologiques de ce micro-organisme sont discutées.