Dr. A. A. B. Sabin  
Children's Research Foundation  
Cincinnati, Ohio.

Dear Dr. Sabin:

Dr. Bauer told me that you are interested in knowing the present status of our observations of pleuropneumonia-like organisms in patients and how we distinguish these organisms from pleomorphic bacteria.

I am glad to write you because I believe that personal contact between those who are interested in a subject is the most helpful method to clear up controversial points. I usually give the enclosed mimeographed description to those who inquire about my method. I delayed its publication because I wished to collect representative photographs and I have not had time to make enough of them. I am very much interested to know the impression which the enclosed photographs will make upon you. I have found PPLO in many cases which probably would have been missed by using Klieneberger's technique or by cultivation in liquid media which you recommended. The success is due to the technique used in examining the cultures. The growth often remains so small and the cultures die out so easily that only by direct microscopical examination of the original plates can they be identified. The morphology of the organisms and the structure of the colonies is so characteristic if they are examined with oil immersion that their classification rarely remains in doubt.

In my opinion the similarity in morphology of these organisms does not indicate their close relationship in the system of bacteria but it indicates a specialized growth form. We probably disagree on this point.

We have observed PPLO in women in about one-third of the cases examined. They were present in some cases without evidence or history of disease. A part of these organisms probably in the same relationship to bacteria as the L. is to the Streptobacillus m. PPLO possess some pathogenicity in females. They are occasionally present in suppurative processes starting in the genitalia, usually in connection with some other bacteria. We have not observed the Reiter's syndrome in females.

All cases in which PPLO were observed presented some pathological symptoms. The most reliable control group in males is constituted by patients with G.C. infection before and after treatment. In females the incidence of PPLO is about the same with or without G.C. We have not found a single male in our hospital who harbored PPLO in cases of male
in our hospital who harbored PPLO in addition to G.C. In a Marine detachment in which many young men had recurring chronic non-specific urethritis three who were infected with G.C. also had PPLO. Salaman's observations on the occurrence of PPLO in cases of male G.C. infections is not reliable because he does not distinguish the PPLO from autolized G. C. colonies or from colonies altered by penicillin. Examination of the urethra of healthy males and of the discharge during and following G.C. infection indicates the PPLO do not belong to the usual flora of the male urethra.

Our recent tabulation comprises 73 patients with positive cultures. The largest group, 30, had nonspecific urethritis and prostatitis. In some only a slight transient discharge was present and the cultures soon became negative. In others the discharge was abundant and lasted with varying intensity for long periods. One patient had a discharge for 14 years. In many cases both the urethral and prostatic secretions PPLO were present in pure culture.

In another group of patients the infection extended to the bladder. Hematuria was present with superficial ulcerations of the mucosa in several cases. The PPLO were present in pure culture in the urine in seven cases. In three others a few colonies of various bacteria probably originating from the urethra grew out in the plates. The presence of PPLO in pure culture in the urine is the best evidence for the pathogenicity of these organisms. They disappeared from the urine in those cases in which treatment with streptomycin was clinically effective.

There is another group (seven cases) of chronic urinary infections in which PPLO were associated with other bacteria. In these cases the significance of the PPLO in relation to the infection cannot be decided.

In the remaining twenty-six cases chronic or acute arthritis was present. Nine presented the Reiter's syndrome of urethritis, conjunctivitis and arthritis. In two of these cases severe cystitis with hemorrhage was present. In 9 other cases conjunctivitis was absent and the genito-urinary symptoms were of variable intensity, sometimes being only very slight. In 8 cases in which chronic arthritis was present, the connection with the genito urinary infection was not clear. The intensity of acute arthritis arthritis varied greatly. Some of the patients had recurring attacks incapacitating them for long periods. Out most striking case is a young doctor who developed urethral discharge after exposure and while under treatment with sulfadiazine and penicillin he developed a hemorrhagic cystitis and a severe polyarthritis. The urine showed PPLO in pure culture in all examinations. In a culture made from one joint fluid two small colonies of the same organism were seen. I have seen rare colonies of PPLO in the joint fluid cultured from one other case. The cultures for PPLO were positive from the urinary tract of about 50% of the Reiter's cases appropriately studied.

PPLO apparently produces often in males a slight infection of the urethra and the prostate with a tendency to chronicity. Infection can extend to the bladder where a violent acute or a chronic infection with periods of exacerbation is produced. In a large percentage of the cases (20-30 percent), acute arthritis is associated with the genito-urinary infection.

We tried agglutination and complement fixation tests with the patients sera and also skin tests. Thus far the results have been negative. We
Photographs I A, B, C, D show a culture from prostatic secretion. 

A.  

With low power shows the tiny colonies and the pus on the agar. The colonies are crowded on the densely inoculated streak, the more sparse away from it. B. shows the surface of the agar with the pus cells on the densely inoculated streak. There are dark masses between the cells but the colonies are not apparent. They become apparent in C, which was made with lowered focus. They are situated below the pus cells embedded in the agar. D. shows a part of a streptococcus colony and the tiny pleuropneumonia-like colonies in an uncrowded area. (x 200)

The colonies in this case did not develop to large size. They would have been missed in an impressive preparation. They are similar in every respect to the colonies of shock strains after about 12 hours of incubation.

Photograph 2 A and B. (X 200)

A and B show a somewhat larger colony of pleuropneumonia-like organisms situated between two small diphtheroid colonies. In A, the focus is on the surface of the agar and the diphtheroid colonies which grow on the surface are clearly visible. The pleuropneumonia-like colony remains blurred. In B, the focus is lowered. The diphtheroid colonies are blurred and the pleuropneumonia-like colony is apparent.

Photograph 3 A, B, and C.

These represent a culture from uterine cervix with low and high magnification. The surface of the pleuropneumonia-like colonies is transformed into large bodies. When the focus is set on the surface of the agar (3 B) the large bodies are entirely similar to the large bodies developing occasionally in gonococcus, H. influenzae or other bacterial colonies. It is characteristic of the pleuropneumonia-like organisms that the growth penetrates into the agar. This is apparent when the focus is lowered (3 C). If photographs 3 C and 1 D are compared it is apparent that the small colonies in 1 D are similar in structure to the part of the larger colonies growing into the agar. Growth stopped in 1 D at an early stage in the development of the colony.

Photograph 4 A, B, C and D are made from a cervical culture. A (low magnification) show large and medium sized L colonies. The tiny colonies are diphtheroids. B and C show large colonies with moderately swollen organisms. D shows a medium sized colony. I call attention to how clearly the morphology of the diphtheroids is apparent, more so than in a dried preparation. This is apparent also in the cocci in 3 B and of the streptococcus and filamentous bacillus in 5. If one becomes familiar with wet preparations, dried preparations, including those made with Klöneberger's technique, appear distorted and blurred.
observed that the strains isolated from the patients were not serologically homogeneous. I have been intrigued very much by the observation that PPLO appear in the sputum in cases with chronic lung infection, if they are treated intensively with penicillin. It is very probably that they develop from H. influenzae because H. influenzae exposed to penicillin in vitro produce similar forms. I have seen tiny PPLO colonies in the cultures of a freshly isolated typhoid strain and of a meningococcus when they were exposed to penicillin. Does it seem likely to you that all these bacterial strains carry a symbiont?

I hope we will have an opportunity to discuss these problems together.

Sincerely yours

Louis Dienes, M.D.