Dear Doctor Sabin:

It was interesting to read your articles because they showed me what you regard important.

I noticed that my photographs made little impression on you. My purpose with them was to test out whether they are good enough to show that agar cultures, if appropriately studied, are much more characteristic for identification of P.P.L.O. than broth cultures. In my opinion, the methods which you recommend for culture and identification of P.P.L.O. are not appropriate in the case of human specimens. You base not only the cultures, but also the final identification on broth cultures. To obtain these is a tedious and long procedure; and in the most interesting cases, like cystitis or Reiter's syndrome, I almost never succeeded in obtaining growth in broth. Transplants were often unsuccessful also on agar and died out after a few generations. The only promising liquid medium is Maitland's medium. The procedure that you recommend would miss almost all cases of serious infection. A further objection which I have against using broth cultures for identification of the strain is that in Giemsa preparations made from broth usually the organisms are entirely distorted. What appears characteristic in these preparations are not the actual organisms but grossly distorted forms.

Identification of the cultures as it is practiced today is often unsatisfactory. I examined the culture of Wallerstein, Vallee, and Turner, which they used for agglutinations. It was not a P.P.L.O. Klieneberger's method is evidently subject to error, though she never made one, but Salaman, working with the same method, in my opinion, mistook bacterial colonies altered by penicillin for P.P.L.O. The use of broth cultures for identification
of the strains, instead of diminishing, would increase the difficulties. Improvement can come only from a method applicable to all cultures and showing clearly the distinctive properties of the cultures. The methods which I worked out in the course of years satisfy these requirements. It takes usually two to three months until one feels confident in making identification of the colonies; but there is no laboratory procedure which is satisfactory without training. It is a matter of observation that cultures appropriately studied are characteristic enough to permit their identification, as in the case of diphtheria bacillus or amoebic cysts. It has nothing to do with theoretical considerations.

Concerning the connection of L1 and streptobacillus, in my opinion the significant fact should be mentioned that a similar connection between bacteria and P.P.L.O. was observed in several other species. I sent to Klieneberger a bacteriodes strain in which the development of L colonies from single bacteria can be easily followed. She observed this process but she tried to maintain her former explanation in somewhat modified form, namely that the L was an intracellular parasite of the bacillus.

The influence of penicillin consists only partially in the inhibition of bacterial growth. It favors evidently also the development of L colonies. For instance, if cultures of the formerly mentioned bacteriodes strain are transplanted from one agar culture to another, none or only a few L colonies develop. As little as 1 unit of penicillin in a cc. of medium prevented the growth of bacteria. In plates containing 1, 5, 10, 50, 100 units per cc., no growth and no L colonies which in transplant grew as well without as with penicillin. Some strains of H influenzae present the same phenomenon. Low concentration of penicillin inhibited all growth. Above a higher, critical concentration, L colonies developed. A synthetic drug which I have started to study apparently has a similar effect on bacteria. In these cases, penicillin or the drug evidently does more than to give a chance to the L by suppressing the bacterial growth.

I noticed a few small errors in the paper. On page 3 in the last paragraph, "the etiological agent for a long time regarded as a filtrable virus and etc"; if I remember well, filterability of the agent was established only one or two years before cultivation, and the concept of viruses at that time was quite hazy.

On page 3 it could be mentioned that P.P.L.O. grew from sterile cystitis.

Page 6, 5th line, "the agar fixation" is connected with my name. This is Klieneberger's method. My method is "agar staining" or "stained agar preparation".
Page 26, line 12, you say that growth on solid media consists predominantly of large plastic protoplasmic masses. This is misleading because young colonies and the central mass of large colonies consist of very small forms. Emphasis on large forms is a consequence of Klieneberger's staining technique which shows usually only the surface of large colonies.

Page 13, concerning the occurrence of P.P.L.O. in throat I mentioned (Process, Soc. Ex. Biol. and Med. 1947, 64, 165) that in 14 throat cultures, using penicillin and anaerobic incubation, 10 were positive for P.P.L.O.

On the same page it is unlikely that Brown's G.C. cultures were anything but occasional mixtures of the two organisms.

In the course of years we got much nearer to each other with Klieneberger concerning the actual observations. I hope that it is only a question of time that we may reach a generally accepted agreement concerning the facts. The attempted interpretations are not without importance because they influence the direction in which we look forward.

I was very sorry that I was not able to attend the International Congress.

With best regards,

Sincerely yours,

Louis Dienes

Louis Dienes, M.D.