Conclusion. The embryonic duck heart, similar to the embryonic chick heart was found to exhibit sensitivity to the action of a digitalis glycoside as characterized by alteration in rate, rhythm and force of contraction. Moreover the embryonic duck heart was able to be used to detect as little as one two-hundredth of a microgram of the digitalis glycoside (Lanatoside C.) This is thought to represent the most sensitive indicator now available for the presence of a digitalis glycoside.

15733 P

Isolation of Pleuropneumonia-like Organisms from Pathological Specimens with the Aid of Penicillin.*

L. DIENES.

From the Department of Pathology and Bacteriology, the Massachusetts General Hospital, and the Robert W. Lovett Memorial Foundation, Harvard Medical School, Boston, Mass.

Pleuropneumonia-like organisms (P.P.L.O.) were isolated from the genito-urinary tract of human patients and in rare cases from other locations.[1-5] Such organisms are often present in the vagina or cervix without apparent connection with any disease. In males they occur only in pathological conditions, and, according to observations collected in the last few years, they play a noticeable role as causative agents in urethritis, prostatitis and cystitis. They have been isolated from cases of severe cystitis which were repeatedly negative for the usual bacteria. Infection of the genito-urinary tract was complicated in about 30% of the cases with acute polyarthritis. It is essential in studying the role of these organisms in human disease that the methods used for their culture and identification be free from error.

The presence of P.P.L.O. can be proven only by cultivation. They grow on media enriched with human or animal serum or with ascitic fluid. Large colonies (0.1-0.3 mm) can be identified with the low power of the microscope. If the colonies remain small (.01-0.1 mm) as often happens in cultures from pathological specimens, they cannot be identified by this method. The agar fixation method which Klieneberger[4] and Salaman[5] used utilizes the impression left by the culture on a coverslip. Small colonies do not adhere to the glass and can not be recognized. This method is subject to error even in the case of large colonies. They are identified by the round bodies which develop on the surface layer and it is not unusual for bacteria to produce similar large bodies. The most reliable method for the identification of the P.P.L.O. is the use of stained agar preparations.[6] A square of the agar culture is cut out and is then covered with a coverslip carrying the stain. The colonies are present in their entirety in the preparations and can be identified with certainty.

Demonstration of P.P.L.O. is usually not possible in the presence of abundant bacterial growth. The P.P.L.O. are resistant to sulfonamides and to penicillin both of which were recommended to suppress bacterial growth. Salaman used penicillin cups for this purpose.[5] The author can confirm the

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1 Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 470.

2 Dienes, L., and Smith, W. E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 99.

3 Beveridge, W. J. B., *Med. J. Australia*, 1943, **2**, 479.

4 Klieneberger-Nobel, E., *The Lancet*, 1945, **2**, 46.

5 Salaman, M. H., and collaborators, *J. Path. Bact.*, 1946, **58**, 31.

6 Dienes, L., *J. Inf. Diseases*, 1939, **65**, 24.

effectiveness of penicillin, but without necessary precautions its use leads to error and will confuse the study of P.P.L.O.

One source of error is that in the area of inhibition, the colonies may remain very small, and the organisms may swell up into large round bodies. They can not be distinguished in an impression preparation from colonies of P.P.L.O. It is probable that the colonies which Salaman identified as mixed colonies of Gonococci and P.P.L.O. were such altered Gonococcus colonies. The author has never seen P.P.L.O. and Gonococcus together in cultures from male patients.

This source of error can be eliminated by using stained agar preparations in which penicillin does not interfere with the identification of P.P.L.O.

The second source of error is the fact that P.P.L.O. can develop from bacteria under the influence of penicillin. It has been shown in another paper that this occurs in pure culture of *H. influenzae*.[7] From a series of 14 throat cultures which were recently studied, P.P.L.O. developed in 10 in the vicinity of the penicillin cups. The distribution of the colonies indicated that they were produced by the penicillin. They were situated in the area of inhibition and were absent in the area not exposed to penicillin. Colonies of P.P.L.O. never develop in throat cultures without the use of penicillin, while they grow very well together with bacteria in cultures from the genito-urinary tract.

According to these observations, penicillin can be used only to screen the specimens for the presence of P.P.L.O. In order to be sure that they are present in the specimens and are not produced from bacteria, their colonies must be seen also in cultures not treated with penicillin. The P.P.L. strains isolated from human patients form a heterologous group, and it is important to distinguish those which are connected with bacteria from those which, like the animal pathogens, do not show such connection.

Summary. Penicillin is of considerable help in isolating pleuropneumonia-like organisms from specimens contaminated with bacteria. Penicillin alters the colonies of certain bacteria in such a manner that in impression preparations they become indistinguishable from pleuropneumonia-like organisms. Furthermore penicillin may induce the growth of pleuropneumonia-like organisms from bacteria. To prove that these organisms are present in the specimens, the characteristic colonies must be apparent in the cultures without the use of penicillin.

[7] Dienes, L., PROC. SOC. EXP. BIOL. AND MED., in press.

15734 P

Isolation of Pleuropneumonia-like Organisms from *H. influenzae* with the Aid of Penicillin.*

L. DIENES.

From the Department of Pathology and Bacteriology, the Massachusetts General Hospital, and the Robert W. Lovett Memorial Foundation, Harvard Medical School, Boston, Mass.

It has been previously noted[1] that certain strains of *H. influenzae* show a pleomorphism similar to that observed in *Streptobacillus moniliformis*[1] and bacteroides.[2] The bacilli swell up into large round bodies which either disintegrate into bacteria of usual appear-

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[1] Dienes, L., *J. Bacteriology*, 1942, **44**, 37.

[2] Dienes, L., and Smith, W. E., *J. Bacteriology*, 1944, **48**, 125.