

STUDIES ON THE PNEUMOCOCCUS.

IV. EFFECT OF BILE AT VARYING HYDROGEN ION CONCENTRATIONS ON DISSOLUTION OF PNEUMOCOCCI.

BY FREDERICK T. LORD, M.D., AND ROBERT N. NYE, M.D.

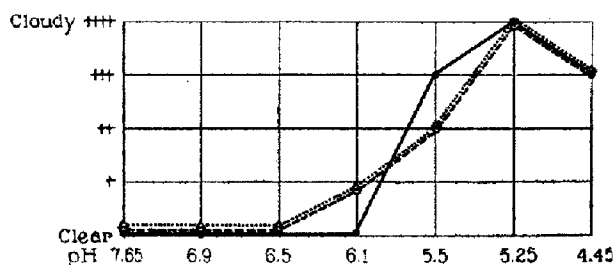
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Fresh beef bile, obtained from the slaughter house, after sterilization for 15 minutes in the autoclave, was found to have a pH of 7.8. By the addition of increasing amounts of HCl to different lots of this bile a series was made with a pH range of 7.65 to 4.45 as determined by the dialysis method (Table I). 4 drops from each of the series of tubes containing bile at varying pH were removed to four sets of seven test-tubes. To one set were added 4 drops of a suspension of *Pneumococcus* Type I, to a second an equal amount of a suspension of Type II, and to a third a suspension of Type III. The fourth set was reserved as a control, 4 drops of distilled water being added to each tube in place of the suspension of pneumococci. After incubation, inspection of the four different series showed the same result in all. As indicated in Text-fig. 1, the tubes containing the three fixed types of pneumococci showed clearing at pH 6.1 and the more alkaline end of the scale, and cloudiness in the more acid end of the scale. The control series without pneumococci showed cloudiness in the acid end of the scale due to precipitation of the bile solution at acidities greater than pH 6.1.

Microscopic examination of smears made from the cloudy tubes showed many masses of amorphous material and many pneumococci, for the most part Gram-negative, a considerable number of Gram-positive organisms, and the shadowy remains of others, while in the clear tubes only a few Gram-positive organisms were found with the shadowy remains of others.

Comparative tests with bile and standard pH solutions show that pneumococci undergo more rapid dissolution in bile. The dissolution in bile may be complete within an hour and only partial in standard solutions within this interval. Dissolution of pneumococci occurs



TEXT-FIG. 1. Dissolution of pneumococci in bile at varying hydrogen ion concentrations. Test-tubes containing 4 drops of bile at varying hydrogen ion concentrations plus 4 drops of suspension of living pneumococcus. Type I, solid line; Type II, dotted line; Type III, broken line. The observations were made after 24 hours in the incubator.

TABLE I.
Method of Preparing Bile at Varying Hydrogen Ion Concentrations.

Tube No.	Bile.	Amount of HCl (half concentrated).	pH	pH after sterilization, filtration, and resterilization.
1	Sterile bile.	0	7.8	7.65
2	25 cc. of A.*	3		6.9
3	25 " " "	6	7.3	6.5
4	25 " " "	7		6.1
5	25 " " B.	6	5.85	5.5
6	25 " " "	10	5.35	5.25
7	C	0	4.9	4.45

* A, fresh bile at pH 7.8 after sterilization; B, bile at pH 6.85, obtained by adding 20 drops of concentrated HCl to 100 cc. of A; C, bile at pH 4.9, obtained by adding 80 drops of concentrated HCl to 100 cc. of A.

most rapidly in the more alkaline end of the bile scale and with the lapse of time is progressive toward the more acid end, becoming complete within 24 hours at a pH of about 6.0 to 7.8 inclusive. Dissolution in standard solutions, however, takes place most quickly at a pH

of about 5.0 to 7.0 and then progresses toward the more alkaline end of the scale. As in standard solutions, dissolution in bile does not take place at the most acid end of the scale.

Dissolution of pneumococci takes place in human as well as beef bile and at the same range of pH. Pneumococci allowed to grow and die out in glucose bouillon, washed, and suspended in bile at varying pH show some but less dissolution than when living organisms are used in the experiment. Bile solubility of dead organisms takes place within the usual range of pH but more slowly than when living organisms are used.

DISCUSSION.

The solubility of the pneumococcus in bile and in standard solutions may be ascribed to the liberation of an enzyme from the bacterial cell. The more rapid solubility of pneumococci in bile at a slightly alkaline reaction and in standard solution at a slightly acid reaction probably depends on a difference in the physical state of the bacterial cell under the influence of the two media. Bile itself probably has the property of killing the pneumococcus with a minimum of injury to the bacterial cell membrane and thus enables the endoenzymes to operate at their optimum reaction which Avery and Cullen¹ have shown to be between pH 7.0 and 7.8. Dissolution in standard solutions on the other hand, proceeds most rapidly at a slightly acid reaction because in this medium death of the organisms, which is dependent on the concentration of hydrogen ions, takes place at this reaction with a minimum of injury to the bacterial cell membrane. The more rapid dissolution in bile than in standard solutions is probably due to the more rapid death of the organisms in bile and hence the liberation of a larger amount of enzyme at the optimum pH in bile than in standard solution.

CONCLUSION.

Dissolution of pneumococci takes place most rapidly in bile at a slightly alkaline reaction. This is probably due to death of the organisms and activation of the endocellular enzyme at its optimum hydrogen ion concentration.

¹ Avery, O. T., and Cullen, G. E., *J. Exp. Med.*, 1920, xxxii, 547, 571, 583.