THE RELATION OF THE PNEUMOCOCCUS TO HYDROGEN ION CONCENTRATION, ACID DEATH-POINT, AND DISSOLUTION OF THE ORGANISM.*

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Lord[1] has called attention to the probable importance of an increase in the hydrogen ion concentration in the pneumonic lung in inhibiting the growth of the pneumococcus and favoring enzymatic action. In this article we wish to elaborate certain aspects of the relation of the pneumococcus to changes in acidity. The hydrogen ion concentrations were determined by the colorimetric method with standard solutions made according to Clark and Lubs' directions.[2] As indicators, phenolsulfonephthalein was used on hydrogen ion concentrations ranging from 8.0 to 6.2, and sodium alizarin sulfonate from 6.0 to 4.0. Colored solutions were either dialyzed or the determinations made by the comparator rack method.

Relation of the Pneumococcus to Varying Hydrogen Ion Concentrations.

Experiment 1.—A 1 per cent glucose bouillon culture of Pneumococcus Type II with an initial hydrogen ion concentration of 7.65 shows an increasing multiplication of the organism, as indicated in Text-fig. 1, and reaches a maximum of growth in about 12 hours. The acidity increases to a pH of 5.25 at which there is a rapid fall in the number of living pneumococci. With the increase in the acidity to a pH of 5.15 no living organisms remain in the flask.

Reinoculation of such a flask in which the pneumococcus has grown and died out and the removal of samples at intervals show that living

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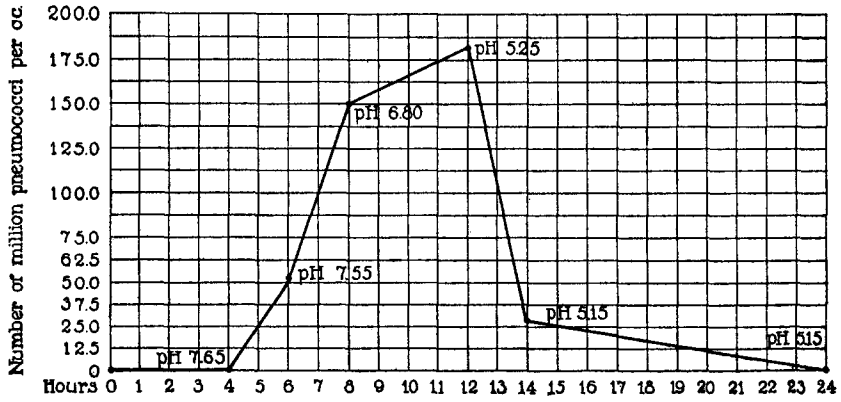
[1] Lord, F. T., *Tr. Am. Soc. Clin. Investigation*, 1916, 8; *J. Am. Med. Assn.*, 1916, lxvii, 1981; 1919, lxxii, 1364.

[2] Clark, W. M., and Lubs, H. A., *J. Biol. Chem.*, 1916, xxv, 479.

organisms can be obtained from the flask for an interval of 1 hour and that no growth is obtained on the transplants taken on and after 3 hours. This experiment suggests that a hydrogen ion concentration of about 5.15 may be regarded as the degree of acidity which will almost immediately kill the organism, but the suggestion is open to the objection that other factors than the acidity may be present and exert a bactericidal action.

Other strains of pneumococci of Types I, II, and III, allowed to grow in 1 per cent glucose bouillon until the culture becomes sterile,



TEXT-FIG. 1. The acid death-point of Pneumococci Type II. The results with 1 per cent glucose bouillon are shown.

produce a final acidity of about the same hydrogen ion concentration. No noteworthy difference among the three fixed types has been noted, with the following exception. One strain of Type I pneumococcus obtained from a fatal case of pneumonia remained alive for at least 10 days after the culture had reached a hydrogen ion concentration of 4.5.

Experiment 2.—Repeated reinoculation of the flask (Experiment 1) after the addition of a sufficient amount of sodium hydroxide to neutralize the titrable acidity results in growth for progressively longer intervals as shown in Table I. Partial exhaustion of the material from which the acid is formed may be regarded as the explanation of the increased viability of the culture.

The experiment suggests that the acidity is the most important factor in the death of the organism since without other change in the conditions of the experiment than the neutralization of the acidity and the lapse of time, the pneumococcus will repeatedly grow in the same flask and for progressively longer periods. However, some other factor than the acidity may also play a part in the death of the organism.

TABLE I.

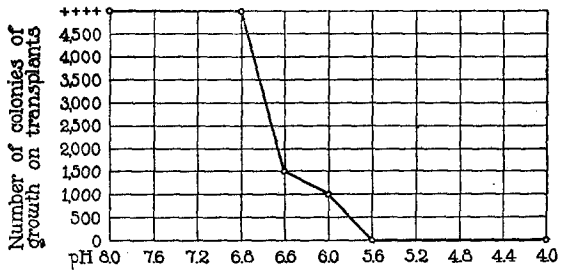
Growth of Pneumococcus in Realkalinized Glucose Bouillon.

Inoculation.	pH	N NaOH required to neutralize 5 cc.	Duration of growth.
		cc.	
1st	7.65–5.15	0.22	14–24 hrs.
2nd	–5.9	0.17	4 days.
3rd	–6.9	0.115	6 "
4th	–7.0		22+ "

1 per cent glucose boullion inoculated with Type II pneumococcus allowed to die out and repeatedly realkalinized and reinoculated.

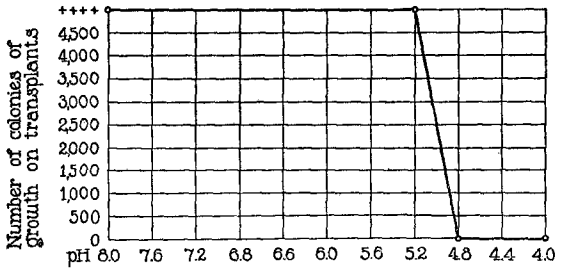
Experiment 3.—In order to test the relation of the pneumococcus to the acidity of the media and to exclude as far as possible the presence of inhibiting substances arising as a result of the growth of the organism, suspensions of washed living pneumococci were made in standard solutions of known hydrogen ion concentrations as follows: Ten drops of each of Clark and Lubs' solutions were placed in small tubes and sterilized in the Arnold sterilizer. Ten drops of a suspension in normal saline solution of the bacterial sediment washed with normal saline solution from an actively growing culture of the pneumococcus were added to each of the solutions of varying hydrogen ion concentrations. The culture of the pneumococcus (Type II) was at the height of its growth and there was no evidence of agglutination or sedimentation in the flask. The addition of the bacteria made a slightly cloudy suspension. The hydrogen ion concentration was not changed by the procedure. The tubes were placed in the incubator and transplants made to the surface of blood serum after 5½ hours, by smearing one loop of the material on the surface, with the result indicated in Text-fig. 2. No growth of organisms was obtained from the tubes at 5.6 or higher hydrogen ion concentrations. An abundant growth of colonies, too numerous to count, was obtained from the tubes at 6.8 and lower hydrogen ion concentrations. About 1,000 colonies were obtained from the tube with a pH of 6.0 and 1,500 from that with a pH of 6.6.

The experiment suggests that irrespective of any bactericidal substances which may be formed in culture media in consequence of the bacterial growth, the pneumococcus will not live for 5½ hours in the presence of hydrogen ion concentrations from 5.6 to 4.0 under the conditions of the experiment, and that some inhibition is present in concentrations from 5.6 to 6.8, beyond which, toward the alkaline end of the scale, more living organisms are present.



TEXT-FIG. 2. The acid death-point of Pneumococci Type II. Suspensions were made in normal saline solution of washed pneumococci at different hydrogen ion concentrations. Transplants were made from these solutions after 5½ hours in the incubator.

Experiment 4.—In Text-fig. 3 the result of a similar experiment with Type III pneumococcus is graphically presented. Bactericidal action of hydrogen ion concentrations from 4.8 to 4.0 acting for 1 hour is indicated.



TEXT-FIG. 3. The acid death-point of Pneumococci Type III. Suspensions were made in normal saline solution of washed pneumococci at different hydrogen ion concentrations. Transplants were made from these solutions after 1 hour in the incubator.

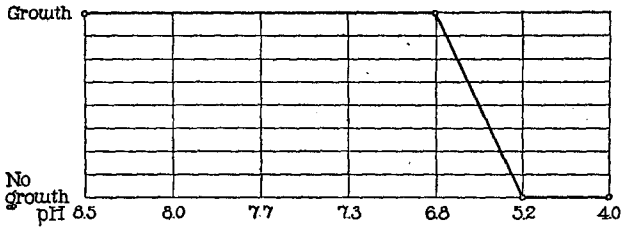
Experiment 5.—To avoid the possibility that the particular solutions used in these experiments (Nos. 3 and 4) may have influenced the result, the experiment was repeated using peptone solutions,[3] of the composition indicated in Table II, of hydrogen ion concentrations varying from 8.5 to 4.0. A large amount of suspension of washed pneumococci (Type II) was added to small amounts of these solutions. After 19 hours in the incubator growth was obtained in transplants from the solutions with a pH of 8.5 to 6.8 as shown in Text-fig. 4. No growth was

TABLE II.

Composition of Solutions for the Determination of the Acid Death-Point.

20 cc. of 4 per cent peptone (Witte's) + hydrochloric acid or sodium hydroxide + 0.5 M phosphates + water to 90 cc.

Flask No.	0.1 N HCl	0.1 N NaOH	0.5 M phosphates.		pH
			KH_2PO_4	Na_2HPO_4	
	cc.	*cc.*	*cc.*	*cc.*	
1	16.0		10.0		4.0
2	8.0		9.0	1.0	5.2
3	3.0		5.0	5.0	6.8
4			2.0	8.0	7.3
5		3.0	1.0	9.0	7.7
6		8.0	0.5	9.5	8.0
7		14.0		10.0	8.5



TEXT-FIG. 4. The acid death-point of Pneumococci Type II. 7.5 cc. of suspensions of washed pneumococci in normal saline solution were added to 2.5 cc. of peptone solutions at hydrogen ion concentrations from 8.5 to 4.0 obtained by adding varying amounts of 0.1 N hydrochloric acid or 0.1 N sodium hydroxide and 0.5 M phosphates. Incubation was for 19 hours. Growth was obtained on transplants from solutions with pH 8.5 to 6.8 and no growth with pH 5.2 to 4.0.

[3] The composition was suggested by Dernby's work (Dernby, K. G., *J. Biol. Chem.*, 1918, xxxv, 179).

obtained from those at 5.2 and 4.0. The experiment indicates that in solutions at a pH of 6.8 and a lower hydrogen ion concentration Pneumococcus Type II will live for 19 hours.

While it is apparent from these experiments that the acidity of the medium has an important bearing on the death of the pneumococcus, it is desirable to obtain data on the relation of the time of exposure to the death of the organism.

Experiment 6.—For this purpose weak solutions of bouillon at varying hydrogen ion concentrations were prepared according to the method indicated in Table III. 5 cc. from each flask were removed to sterile test-tubes. The tubes were

TABLE III.

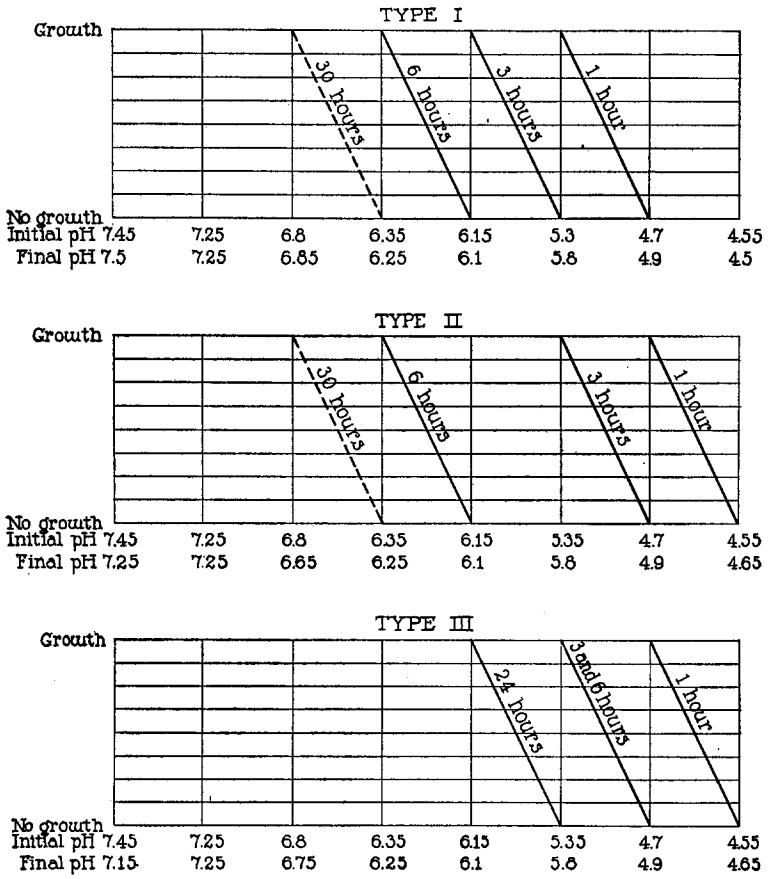
Composition of Solutions for the Determination of the Acid Death-Point.

20 cc. of plain bouillon, hydrochloric acid, and 0.5 M phosphates + water to 90 cc.

Flask No.	0.1 N HCl	0.5 M phosphates.		pH
		KH_2PO_4	Na_2HPO_4	
	cc.	*cc.*	*cc.*	
1	12.0	10.0	0.0	4.55
2	10.0	9.5	0.5	4.7
3	8.0	9.0	1.0	5.35
4	6.0	7.5	2.5	6.15
5	4.0	6.5	3.5	6.35
6	2.0	4.0	6.0	6.8
7	0	2.0	8.0	7.25
8	0	0.0	0.0	7.45

set up in triplicate and one drop of a suspension in salt solution of washed pneumococci of Type I, II, or III was added to each tube of the three series. The tubes were then placed in the incubator and transplants made on blood serum at intervals of 1, 3, and 6 hours. It is apparent from the graphic presentation of the results in Text-fig. 5 that there is a time element in the bactericidal action of acidity. Thus Type I was killed by exposure to a hydrogen ion concentration of 4.5 and 4.7 for 1 hour, but survived an acidity of 5.3 for this interval only to succumb to it after exposure for 3 hours. A pH of 6.15 was withstood for 3 but not for 6 hours and living organisms were obtained after this interval at a pH of 6.35. Type II showed slightly less susceptibility to acid in this experiment. Type III is still less susceptible. Other experiments indicate that there is no constant difference in the acid death-point of the fixed types. Determination of the hydrogen ion concentrations of the mixtures before the experiment and 24 hours later showed no noteworthy change.

From these and other experiments it may in general be said that in fresh culture media the pneumococcus withstands a pH of about 5.3 for 1 hour, of 5.6 for 3 hours, and of 6.1 for 6 hours. It is to be noted (Experiment 1) that when reinoculated into culture media in which the organism has grown and died out it survives a pH of about 5.1



TEXT-FIG. 5. The relation of time and acidity to the death of pneumococci. Suspensions of pneumococci in normal saline solution were added to bouillon solutions at hydrogen ion concentrations varying from 7.45 to 4.5. Transplants were made after varying intervals. The 30 hour periods in Types I and II are taken from another similar experiment.

for 1 hour, thus showing by comparison that there is no essential difference in the acid death-point in fresh culture media and that in culture media containing whatever products may be liberated by the growth and death of the organisms. The acidity is therefore the most important factor.

In contrast to the preceding experiments the following experiments indicate that cultures of pneumococci remain viable when the acidity is low.

Experiment 7.—1 per cent glucose calcium carbonate bouillon with an initial pH of 7.4 was inoculated with pneumococci June 10, 1915. Transplants every few days to June 28 showed growth. On June 28 the hydrogen ion concentration was 6.7. Transplants at less frequent intervals showed growth through December 15. The hydrogen ion concentration was not again determined. The total duration of life was about 6 months.

Experiment 8.—Plain bouillon with an initial pH of 7.4 was inoculated with pneumococci June 10, 1915. Transplants every few days to June 28 showed growth. The pH was then 7.4 as before. Further transplants gave growth through December 15. The hydrogen ion concentration was not again determined. The total duration of life was about 6 months.

Experiments 6 to 8 suggest that within the range of hydrogen ion concentrations investigated (pH 7.45 to 4.5) the pneumococcus is killed by hydrogen ion concentrations above about 6.8 with a rapidity which bears a direct relation to the hydrogen ion concentration, *i.e.* the greater the hydrogen ion concentration the more rapid the death, and that the pneumococcus will continue to live in suitable culture media at a pH of about 6.8 to 7.4 for at least many days.

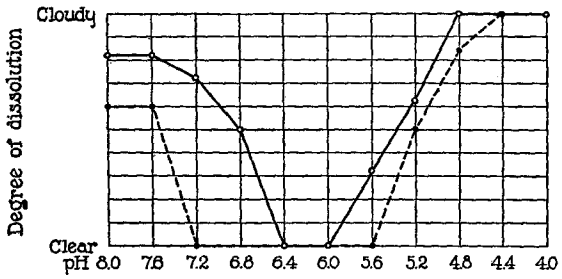
The following experiments suggest that some other factor than the acidity alone is responsible for destruction of pneumococci.

Influence of Varying Hydrogen Ion Concentrations on Dissolution of Pneumococci.

A striking phenomenon which may be spoken of as dissolution is observed when to ten drops of standard solutions of known hydrogen ion concentration ten drops of a suspension of washed pneumococci are added and the resulting slightly cloudy suspension of pneumococci is placed in the incubator. A clearing of the tubes at a hydrogen ion concentration of about 5.0 to 6.0 is observed. The tubes on the more

acid side of the scale remain homogeneously cloudy, although there may be some diminished density at 4.4 and 4.8 and there is constantly some clearing which increases with time on the more alkaline side of the scale. The clearing of the suspension at different hydrogen ion concentrations is graphically indicated in Text-figs. 6 to 8.

Microscopic examination of films from the different tubes shows that the pneumococci in the tubes at the acid end of the scale maintain their morphology, but for the most part become Gram-negative. Examination of films from the cleared suspensions within the range of a pH of about 5.0 to 6.0 shows a few poorly stained organisms, with the shadowy remains of others. The pneumococci are disintegrated and many remnants show as staining points. All are decolorized by



TEXT-FIG. 6. The degree of dissolution of Pneumococci Type I after 7 hours (solid line) and 24 hours (broken line). The suspensions of washed pneumococci in normal saline solution were added to solutions of different hydrogen ion concentrations.

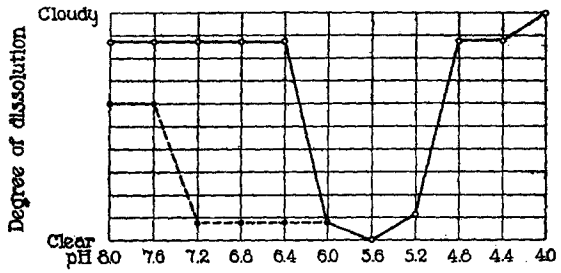
Gram's stain. Films from the tubes at the alkaline end of the scale show some but much less disintegration. Many organisms retain the Gram stain.

The explanation of the dissolution of the organisms is uncertain. It does not seem to be due to the particular chemical substances, sodium chloride, acid potassium phthalate, potassium dihydrogen phosphate, and sodium hydroxide used in this experiment, since the sodium hydroxide is the only variable factor and the curve of dissolution does not correspond to the curve of sodium hydroxide concentration. Moreover, the same curve of dissolution occurs in other standard solutions without sodium hydroxide. The addition of ten drops of

suspension of washed pneumococci to these solutions changes the hydrogen ion concentration little or not at all. Calculation shows that

TEXT-FIG. 7. The degree of dissolution of Pneumococci Type II after 5½ hours. The suspensions of washed pneumococci in normal saline solution were added to solutions of different hydrogen ion concentrations.

a change in the molecular concentration of the solutions is an unlikely explanation. It is not due to the acidity alone, since if it were the acid end of the scale would not remain cloudy. It may be conjec-



TEXT-FIG. 8. The degree of dissolution of Pneumococci Type III after 1 hour (solid line), and 18 hours (broken and solid line). The suspensions of washed pneumococci in normal saline solution were added to solutions of different hydrogen ion concentrations.

tured that some other factor besides the acidity is responsible and the activation of an enzyme derived from the bacteria themselves may be the explanation.

CONCLUSIONS.

1. In the growth and death of the pneumococcus in fluid media containing 1 per cent glucose the production of acid is the most important bactericidal factor.

2. 1 per cent glucose bouillon cultures of the pneumococcus allowed to grow and die out usually reach a final acidity of a pH of about 5.1.

3. At a hydrogen ion concentration of about 5.1 or higher, the pneumococcus does not survive longer than a few hours.

4. In hydrogen ion concentrations of about 6.8 to 7.4 the pneumococcus may live for at least many days.

5. In the intervening hydrogen ion concentrations, between 6.8 and 5.1, the pneumococcus is usually killed with a rapidity which bears a direct relation to the hydrogen ion concentration; *i.e.*, the greater the acidity the more rapid is the death.

6. Cloudy suspensions of washed pneumococci in hydrogen ion concentrations varying from 8.0 to 4.0 show, after incubation, dissolution of organisms in lower hydrogen ion concentrations than about 5.0. This dissolution is most marked at about 5.0 to 6.0. Some dissolution also takes place toward the more alkaline end of the scale. No dissolution occurs at the most acid end of the scale.

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