

STUDIES ON THE PNEUMOCOCCUS.

I. ACID DEATH-POINT OF THE PNEUMOCOCCUS.

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In a previous publication¹ we brought forward evidence indicating that in the growth and death of the pneumococcus in fluid cultures containing 1 per cent glucose the production of acid was the most important bactericidal factor and that such cultures when allowed to grow and die out usually reach a final pH of about 5.1. In our experiments on the relation of the time of exposure to the death of the organisms we made use of weak solutions of bouillon at varying hydrogen ion concentrations. In order to exclude the possibility that an unsuitable medium might play a part in these results, we have since used a stronger broth adjusted to approximate isotonicity, as shown in Table I.

5 cc. from each flask (Table I) were removed to sterile test-tubes and each tube of the series was inoculated with 2 drops of a saline suspension of washed living pneumococci. Transplants were made on blood serum after varying periods of incubation. Table II indicates the results obtained in an experiment with Type I pneumococcus. No noteworthy change in the hydrogen ion concentration of the tubes occurs during the experiment. It will be seen that at a pH of 4.47 no living organisms were obtained after an interval of 1 hour. At a pH of 5.15 living organisms were obtained after 1 hour but not after 2. At a pH of 5.78 organisms were living after 2 hours but not after 3, while at a pH of 6.33 organisms were obtained after 6 hours. At a pH of 6.63 to 7.80 inclusive transplants were positive from 8 to 10 days.

A considerable number of similar experiments has been performed

¹ Lord, F. T., and Nye, R. N., *J. Exp. Med.*, 1919, xxx, 389.

TABLE I.

Isotonic Broth for Testing the Acid Death-Point of the Pneumococcus.

| Flask No. | pH* | 0.5 M KH ₂ PO ₄ cc. | Na ₂ HPO ₄ cc. | HCl | | NaOH (concentrated). cc. | Depression of freezing point. °C. | 10 per cent NaCl. cc. | H ₂ O cc. | Final depression of freezing point. °C. | Final pH.† |
|-----------|------|--|---|-----|---------------|--------------------------------|--------------------------------------|--------------------------|-------------------------|--|------------|
| | | | | N | Concentrated. | | | | | | |
| | | | | T | cc. | | | | | | |
| 1 | 4.9 | 15.0 | | 1 | 0.3 | | 0.450 | 6.7 | 3.3 | 0.620 | 4.47‡ |
| 2 | 5.3 | 15.0 | | 1 | 0.2 | | 0.378 | 7.4 | 2.6 | 0.622 | 5.15 |
| 3 | 5.75 | 15.0 | | 1 | 0.1 | | 0.390 | 8.0 | 2.0 | 0.584 | 5.78 |
| 4 | 6.1 | 15.0 | | | | | 0.322 | 11.4 | | 0.638 | 6.33 |
| 5 | 6.4 | 13.8 | 1.2 | | | 0.3 | 0.378 | 8.4 | 1.6 | 0.598 | 6.63 |
| 6 | 6.9 | 9.45 | 5.55 | | | 0.3 | 0.349 | 9.5 | 0.5 | 0.571 | 7.01 |
| 7 | 7.5 | | 15.00 | | | | 0.328 | 11.0 | | 0.700 | 7.51 |
| 8 | 7.8 | 1.2 | 13.8 | | | 0.1 | 0.305 | 11.0 | | 0.621 | 7.80 |

* Determined colorimetrically.

† Determined electrometrically.

‡ The figure in the hundreds place is uncertain by this method.

TABLE II.

Acid Death-Point of the Pneumococcus. Time Relation at Varying Hydrogen Ion Concentrations.

| Flask No. | pH* | Results obtained on transplants after varying intervals. | | | | | | | | | | | | | Final pH.† | | | | | | | |
|-----------|------|--|--------|--------|--------|--------|--------|--------|---------|---------|---------|---------|---------|---------|------------|---------|---------|----------|----------|----------|------|------|
| | | 1 hr. | 2 hrs. | 3 hrs. | 4 hrs. | 5 hrs. | 6 hrs. | 7 hrs. | 24 hrs. | 48 hrs. | 72 hrs. | 4 days. | 6 days. | 7 days. | | 8 days. | 9 days. | 10 days. | 12 days. | 13 days. | | |
| 1 | 4.47 | 0 | 0 | | | | | | | | | | | | | | | | | | 4.47 | |
| 2 | 5.15 | + | 0 | 0 | | | | | | | | | | | | | | | | | | 5.15 |
| 3 | 5.78 | + | + | 0 | 0 | | | | | | | | | | | | | | | | | 5.72 |
| 4 | 6.33 | + | + | + | + | + | + | 0 | 0 | | | | | | | | | | | | | 6.19 |
| 5 | 6.63 | + | + | + | + | + | + | + | 0 | + | + | + | + | + | + | 0 | 0 | | | | | 6.58 |
| 6 | 7.01 | + | + | + | + | + | + | + | + | + | + | 0 | + | + | + | + | 0 | 0 | | | | 6.58 |
| 7 | 7.51 | + | + | + | + | + | + | + | + | + | + | + | + | + | 0 | + | 0 | 0 | | | | 7.14 |
| 8 | 7.80 | + | + | + | + | + | + | + | + | 0 | + | + | + | + | + | 0 | + | + | 0 | 0 | | 7.30 |

* Determined electrometrically.

† Determined colorimetrically.

‡ 0 indicates lack of growth on the transplant; +, growth.

with Type I and II pneumococci. Though there is slight variation in the time element in the bactericidal action of acidity, the results are essentially the same for the two types and confirm the results previously obtained and reported; at a pH of about 5.1 or lower the pneumococcus does not survive longer than a few hours, at a pH of about 6.8 to 7.4 the pneumococcus may live for at least many days, and in the intervening solutions, between 6.8 and 5.1, the organism 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 the death.

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