

## STUDIES ON THE PNEUMOCOCCUS.

### III. DISSOLUTION OF PNEUMOCOCCI IN PNEUMONIC CELLULAR MATERIAL AT VARYING HYDROGEN ION CONCENTRATIONS. RESISTANCE OF CERTAIN OTHER ORGANISMS TO DISSOLUTION.

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To determine the effect at varying hydrogen ion concentrations of cellular material obtained from the pneumonic lung on pneumococci and other organisms, the following experiments were performed.

The mash from a pneumonic lung due to Type I pneumococcus and preserved with chloroform and toluene was ground in a sterile mortar with some of the saline solution with which the material was originally put up. The resulting cellular material was allowed to stand on ice until the supernatant fluid was clear. The supernatant fluid and sediment were put on Löffler's blood serum. Both produced proteolysis, the cells more than the supernatant fluid. The pH of this fluid was 5.5.

The pH of the cellular material was changed by adding to 10 cc. 9 drops of normal NaOH, which gave a pH of 6.95. To another 10 cc. lot 1 drop of concentrated HCl was added, with a pH of 4.5 as the result.

*Dissolution Experiment with Pneumococci.*—The cellular material of varying hydrogen ion concentrations thus prepared was set up with suspensions of Type I, II, and III pneumococci as shown in Table I. As will be seen from the table the behavior in the cellular suspension at varying hydrogen concentrations was practically the same for the three types. All three types at a pH of 4.5, after incubation over night, for the most part retain their morphology. Type I pneumococcus at this pH remained Gram-positive while Types II and III became Gram-negative. All three types underwent dissolution at a pH of 5.5 and 6.95.

The dissolution of pneumococci taking place in cellular material from the pneumonic lung at a pH of 6.95 and 5.5 may be ascribed to the action of an enzyme operative at this pH. Previous experiments indicate that the enzyme may be derived from the bacteria themselves, since similar dissolution of the organisms at this pH is noted in stand-

TABLE I.  
*Relation of Hydrogen Ion Concentration to Dissolution of Pneumococci in Cellular Material from the Pneumonic Lung.*

| Tube No. | Amount of cellular suspension. |        |         | Amount of pneumococcus suspension. |          |           | Smears.                                                                    |                                                                            |
|----------|--------------------------------|--------|---------|------------------------------------|----------|-----------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|
|          | pH 4.3                         | pH 5.5 | pH 6.95 | Type I.                            | Type II. | Type III. | Made at once.                                                              | Made after incubation over night.                                          |
| 1        | gtt.                           | gtt.   | gtt.    | gtt.                               | gtt.     | gtt.      | Organisms numerous, in good condition, and all Gram-positive.              | Organisms well preserved, in good condition, and Gram-positive.            |
| 2        |                                | 5      |         | 5                                  |          |           |                                                                            | Organisms have disintegrated and disappeared.                              |
| 3        |                                |        | 5       | 5                                  |          |           |                                                                            | Organisms have disintegrated and disappeared.                              |
| 4        | 5                              |        |         |                                    | 5        |           | Organisms well preserved but all Gram-negative.                            | Organisms have disintegrated and disappeared.                              |
| 5        |                                | 5      |         |                                    | 5        |           |                                                                            | Organisms have disintegrated and disappeared.                              |
| 6        |                                |        | 5       |                                    | 5        |           |                                                                            | Organisms have disintegrated and disappeared.                              |
| 7        | 5                              |        |         |                                    |          | 5         | Organisms mostly well preserved, a few slightly eroded; all Gram-negative. | Organisms mostly disintegrated; an occasional Gram-negative organism seen. |
| 8        |                                | 5      |         |                                    |          | 5         |                                                                            | Organisms have disintegrated and disappeared.                              |
| 9        |                                |        | 5       |                                    |          | 5         |                                                                            |                                                                            |

ard solutions or bouillon without cellular material. An enzyme derived from the cellular material may also, however, be a factor in the dissolution.

*Dissolution Experiment with Other Organisms.*—To a cellular suspension from a Type I pneumonic lung with a pH of 5.5, suspensions

of living and washed *Streptococcus haemolyticus* and of *Streptococcus viridans* were added. To another portion of the same cellular suspension, Type I pneumococcus was also added as a control. In setting up the experiment 5 drops of the cellular suspension were added to 5 drops of a suspension of the different organisms. Microscopic examination of the mixtures after incubation over night showed that the pneumococci had disappeared with the exception of an occasional Gram-positive organism. There were many fragments of Gram-positive organisms and many staining points. *Streptococcus haemolyticus* and *Streptococcus viridans* were not dissolved and remained Gram-positive after exposure in the incubator to the cellular suspension.

#### CONCLUSIONS.

Pneumococci of Types I, II, and III undergo dissolution when mixed with cellular material from the pneumonic lung at a pH of 6.95 and 5.5, but not at a pH of 4.5. An enzyme derived from the bacteria themselves or from the cellular material may be the cause of the dissolution. *Streptococcus haemolyticus* and *Streptococcus viridans* do not undergo dissolution under similar experimental conditions.