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Bactericidal Effect of an Extract of a Soil Bacillus on Gram Positive Cocci.

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An unidentified spore-bearing bacillus, capable of causing the lysis of living gram-positive cocci, has been isolated from a soil sample to which suspensions of these cocci had been added over a long period of time. Autolysates of cultures of the soil saprophyte have yielded a soluble factor which lyses living staphylococci, pneumococci (R and S forms, irrespective of type-derivation), hemolytic, green and indifferent streptococci (all types so far tested). The active principle is not volatile, does not dialyze through collodion membranes, and is heat-labile. It is very stable at alkaline reactions, but is rapidly inactivated at reactions more acid than pH 5.5, even at room temperature. When maintained at 0°C, the active principle can be precipitated quantitatively at pH 4.2-4.4, and the precipitate, redissolved in a neutral medium, exhibits the lytic activity of the original solution.

Several tests have been used to investigate the effect of this soluble extract upon the living cells of a number of bacterial species.

1. *Lytic activity.* 1 mg (dry weight) of the bacterial extract, added to 10⁹ pneumococci in a medium at pH 7.5, causes complete lysis of the bacterial cells after 1 hour at 37°C.

Staphylococci undergo solution under the same conditions, but somewhat more slowly. Although lysis of streptococci can also be obtained, it is always less complete and requires larger amounts of extract.

2. *Bactericidal effect.* Pneumococci, streptococci, and staphylococci incubated at 37°C with the extract, are rapidly killed. For instance, 1 mg of extract (dry weight) is enough to kill 10¹⁰ pneumococci or streptococci in 2 hours at 37°C. Similar results have been obtained with staphylococci. The cocci, however, do not lose their viability when the mixtures of extract and bacterial cells is maintained at 0°C even for several hours.

3. *Inhibition of growth.* Minute amounts of extract added to nutrient broth prevent the growth of gram-positive cocci. For instance, 0.0001 mg of extract is sufficient to inhibit the growth of 10⁷ pneumococci inoculated into 5 cc of broth. Inhibition of growth of streptococci and staphylococci requires somewhat larger amounts

of extract. On the contrary, the extract is entirely unable to prevent or retard the multiplication of any of the gram-negative bacilli so far tested, even when the test is carried out with very small inocula (10 bacilli) and large amounts of extract.

4. *Inhibition of dehydrogenase-activity.* Streptococci which have been incubated at 37°C with the bacterial extract, lose the ability to reduce methylene blue in the presence of glucose. In fact, inactivation of the dehydrogenase can be recognized before any morphological alteration of the cocci has taken place; it appears likely, therefore, that lysis is only a secondary process following some injury inflicted upon the cell.

5. *Protective effect against infection of experimental animals.* Intraabdominal injection of the bacterial extract protects white mice against infection with large numbers of virulent pneumococci. A single injection of 2 mg of the extract will protect white mice against 10,000-100,000 fatal doses of pneumococci of Types I, II, III, V, VIII (other types have not been tested). Protection against larger doses requires repeated treatments. The extract also exerts a curative effect when administered to mice several hours after infection with virulent pneumococci. Preliminary studies carried out with Dr. R. C. Lancefield indicate that the extract is also capable of protecting mice against infection with hemolytic streptococci.

Discussion. The results of *in vitro* experiments seem to indicate that the protective action *in vivo* of the bacterial extract is due to a direct bactericidal effect on gram-positive cocci. It is interesting to contrast this direct effect with the mechanism of the protection induced by a bacterial enzyme that hydrolyses the capsular polysaccharide of Type III pneumococcus. As described in earlier studies, this polysaccharidase does not in any way affect the viability of pneumococci; by decomposing the capsular substance, however, it renders the bacterial cells susceptible to destruction by phagocytosis.^{1,2} The polysaccharidase does not attack the specific polysaccharides of other types of pneumococci, and consequently it protects only against infection with Type III organisms. On the contrary, the bacterial extract considered in the present paper inhibits the growth of all gram-positive cocci so far tested and exerts on them a bactericidal effect *in vitro*; its protective action *in vivo* has already been established against several different types of pneumococci and hemolytic streptococci. It is worth emphasizing again, however, that the extract does not affect the viability or inhibit the growth of gram-negative bacilli.

¹ Dubos, R. J., and Avery, O. T., *J. Exp. Med.*, 1931, **54**, 51.

² Avery, O. T., and Dubos, R. J., *J. Exp. Med.*, 1931, **54**, 73.