FURTHER STUDIES OF THE PHENOMENON OF CAPSULAR SWELLING OF *MICROCOCCUS PYOGENES* VAR. *AUREUS* IN THE PRESENCE OF IMMUNE SERUM

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In 1954 we (Price and Kneeland) described a mucoid form of *Micrococcus pyogenes* var. *aureus* (Staphylococcus aureus) which showed capsular swelling with homologous immune serum. This mucoid variant, designated RL-mucoid, was derived from the parent RL strain after passage through embryonated eggs with influenza virus. Attempts to reproduce this variation by passing RL through embryonated eggs on subsequent occasions were unsuccessful. The original RL-mucoid variant, however, could be maintained indefinitely on subculture, provided that an occasional egg passage was performed if the strain appeared to be losing its mucoid character.

RL-mucoid and RL were found to be mannitol- and coagulase-positive, to have similar virulence for mice, and identical phage typing. Apart from the differences in colonial morphology, the two strains could only be distinguished by their capacity to show capsular swelling, and by their capacity to produce potent antisera. In other words, anti-RL-mucoid serum produced rapid and complete capsular swelling of RL-mucoid organisms, whereas only an occasional RL organism would be so affected. Similarly, anti-RL serum caused relatively slight capsular swelling of RL-mucoid organisms, and none of the RL organisms. It was thought that the difference between RL-mucoid and RL was a quantitative one, and related to the amount of capsular substance produced.

In the discussion it was stated that "the serological specificity of this reaction remains to be determined, since we have not had the opportunity to study the capsular substance from a variety of different strains of staphylococci to find out if they are antigenically distinct. If this should be the case, it would create a new method for serological classification." The present communication presents data relating to 39 other strains, and indicates that the reaction is not type-specific.

METHODS AND MATERIALS

The techniques employed in culturing the organisms, demonstrating capsular swelling, producing antiserum, and performing the coagulase tests were described in our previous publication. Alpha toxin was demonstrated by the method of Elek and Levy (1950). Adsorption of antibody from serum was done by the method of Cowan (1939).

The sources of the 39 additional strains of staphylococci studied were as follows: From pathological sources: ulcerative colitis, 8; suppurative ophthalmological conditions, 6; septicemia, 4; urinary tract infections, 2; pneumonia, throat infection, chronic bronchiectasis, breast abscess, sinusitis, and postantibiotic diarrhea, 1 each. In addition, 3 strains were supplied by Dr. Samuel Cowan from the National Collection of Type Cultures, London, England. From normal sources: normal conjunctivae, 10.

RESULTS

Thirty-two of the strains showed capsular swelling when treated with anti RL-mucoid serum. With 25 of these strains, capsular swelling could be demonstrated in the stock broth cultures. In nine instances, egg passage was required to produce the phenomenon. Twenty-eight of these 32 strains came from pathological sources, all produced alpha toxin, and all but two were both mannitol- and coagulase-positive. Of the two atypical strains, one was mannitol-negative and coagulase-positive, the other both mannitol- and coagulase-negative.

Three of these 28 strains from pathological sources were mucoid—two from the time of original isolation, and one after egg passage. Antisera prepared against two of our nonmucoid strains and the three Cowan strains by prolonged immunization produced capsular swelling of the RL-mucoid strain as well as of the homologous

organisms[1]. Eight of the strains from pathological sources were phage-typed, and all showed varying phage patterns.

Six strains, all from normal sources, all coagulase- and alpha toxin-negative, and all but one mannitol-negative, showed no capsular swelling when exposed to anti RL-mucoid serum in spite of three egg passages. Antiserum was prepared against three of these strains by prolonged immunization and showed no capsular swelling of RL-mucoid organisms.

We have encountered only one strain of staphylococcus which produced alpha toxin, and was mannitol- and coagulase-positive, which did not show capsular swelling with anti-RL-mucoid serum. A strain was not recorded as not producing capsular substance until it had been passed three times in eggs.

DISCUSSION

Additional data are presented in regard to the production of capsular material by staphylococci and the phenomenon of capsular swelling when the organisms are exposed to antiserum. The observations indicate that nearly all strains of staphylococci which ferment mannitol and produce coagulase and alpha toxin create a small amount of capsular substance. The rarely encountered mucoid variant, however, produces quantitatively a great deal more of the capsular substance. This reflects itself in two ways: first, mucoid organisms make a better antigen. Strong antiserum can be produced with a relatively short course of immunization and it retains its potency well. Second, mucoid organisms show the "quellung" reaction much more strikingly; the capsules are larger, all the organisms show them, and the reaction is complete in about three hours. With nonmucoid strains only some of the organisms show the phenomenon, the capsules are smaller, and the reaction is scarcely noticeable until the preparation has stood overnight.

That these differences are quantitative rather than qualitative, and that the reaction is not strain-specific, is indicated by the data herewith presented. Anti-RL-mucoid serum produced capsular swelling in 29 of the strains, a number of which showed different phage patterns, and also with Cowan's types I, II, and III organisms. Moreover, antiserum against two of our own nonmucoid strains as well as the three Cowan strains produced the reaction with RL-mucoid organisms. Lastly, the capsular-swelling antibody could be adsorbed from anti-RL-mucoid serum not only by the homologous organisms but by Cowan's types I, II and III. Staphylococci not showing the capsular-swelling phenomenon were incapable of adsorbing it.

Thus the production of capsular substance and the phenomenon of capsular swelling appear to be properties of nearly all "pathogenic" staphylococci, and show a close correlation with mannitol fermentation, and the production of coagulase and alpha toxin. The amount of capsular substance produced does not seem to be related to virulence.

[1] This is in apparent conflict with the statement in our first paper that the phenomenon was not produced by stock antisera. The sera referred to, however, were eighteen years old at the time, and thus may have lost their capsular-swelling property.

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SUMMARY

Most strains of staphylococci which are mannitol- and coagulase-positive and produce alpha toxin also produce capsular substance which can be demonstrated by the "quellung" reaction, using appropriate technique.

The amount of capsular substance produced varies from strain to strain, being much greater in mucoid strains, but is antigenically similar in all strains studied. It does not appear to be quantitatively related to virulence.

Several strains, all isolated from nonpathological sources, which did not produce alpha toxin, and were mannitol- and coagulase-negative, did not give rise to capsular substances.

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