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Production of Small Colony Variants of *Staphylococcus aureus* by the Action of Penicillin.*

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The production of stable small colony variants from one strain of *Staphylococcus albus* by the action of penicillin was reported by Schnitzer, Camagni, and Buck.[1] In view of the fact these workers found their small colony variants to be penicillin resistant it was thought advisable to determine whether penicillin would have the same action on pathogenic strains of *Staphylococcus aureus*.

Methods. Ten recently isolated, hemolytic, coagulase positive, pathogenic strains of *Staphylococcus aureus* were employed. These strains were all purified before exposure to penicillin by 3 successive single colony isolations.

Serial dilutions of a solution of the sodium salt of penicillin sterilized by filtration were made aseptically in tubes containing 5.0 cc of nutrient broth, each dilution being half the preceding one. The concentration of penicillin in the first tube of each series was one unit per cc. Each tube, including suitable control tubes containing no penicillin, was then inoculated with 0.1 cc of a 24-hour broth culture of the organism to be tested. Readings of growth inhibition were made following 24-hour incubation at 37°C and agar plate subcultures were then made from the control tubes and from a sufficient number of tubes in each series of dilutions to overlap, by 2 or 3 tubes, the zone of growth inhibition. These subcultures were repeated at 2- to 3-day intervals over a period of 7 to 10 days. The agar plates were incubated at 37°C and examined daily for a period of not less than 4 days.

Results. Small colony variants were obtained from 9 of the 10 strains in from 2 to 9 days following the initial exposure to penicillin. None were found in the controls. The susceptibility of the 10 strains to penicillin varied considerably and there was no absolute relation between the concentration of penicillin and the production of small colony variants. Instead, these variants always appeared in the concentration of penicillin which markedly inhibited but did not completely prevent growth. They were never found in more than 2 tubes in a series of dilutions. Furthermore, the concentrations of penicillin in which the appearance of small colony variants was most pronounced were usually those in which there was no evidence of growth following 24-hour incubation but which gradually became turbid following further incubation. These findings are similar to those previously reported on the production of small colony variants by inorganic salts.[2]

The number of small colony variants obtained varied from strain to strain and from tube to tube. Sometimes only a few would be found interspersed among normal *Staphylococcus aureus* colonies, at other times nearly all of the growth on a plate would consist of these variants.

All of the small colony variants obtained were of the unstable type and had the typical characteristics previously described for this form.[2] The rapid reversion of these forms made it impossible to test their penicillin sensitivity but the manner of their production would suggest a greater resistance than the normal form of the organism.

These results show that penicillin is an effective agent for the production of small colony variants of *Staphylococcus aureus* and since they appear most readily in concentra-

* Part of the penicillin employed was furnished by the Penicillin Research Committee, Northwestern University Medical School.

1 Schnitzer, R. J., Camagni, Lillian J., and Buck, Margaret, PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 75.

2 Youmans, Guy P., and Delves, Edna, *J. Bact.*, 1942, **44**, 127.

tions of penicillin which are almost completely inhibitory, the results further indicate the advisability of using maximal rather than minimal doses of penicillin for the clinical treatment of staphylococcal infections.

Summary. Unstable small colony variants were readily produced from nine out of ten strains of *Staphylococcus aureus* upon exposure to penicillin.

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Observations on the Antagonism Between Heparin and Trypsin.*

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It has been stated that following anaphylactic shock there may be more than the normal amount of antitrypsin in the blood.[1,2,3] The incoagulability of the blood in anaphylaxis has been explained by the discovery that it contains substantial amounts of heparin,[4] and reports that heparin has antitryptic properties[5,6] have led to the suggestion that this substance may be responsible for the increase in the antitryptic activity of such blood.

We have observed that heparin inhibits the histamine releasing effect of trypsin on rabbit blood cells,[7] and that it protects rats from the lethal effects of intravenously injected trypsin.[8] Our observations were interpreted as supporting the view that heparin has a specific antitryptic action. However, the recent demonstration that the blood of heavily heparinized dogs has no increased antiproteolytic activity[9] necessitates a reconsideration of such a view, and has led to the additional studies reported here on the antagonism between heparin and trypsin.

The intravenous injection of heparin (10-15 mg per kilo) did not modify the fall in blood pressure produced by the intravenous injection of trypsin (0.1-1.0 mg per kilo) into anesthetized dogs (4 experiments). Nor did the incubation of heparin with trypsin for various periods of time prior to injection alter its depressor activity. On the other hand, the trypsin preparation used ("Difco") was inactivated when incubated with blood serum, which is known to be antitryptic. These results, therefore, seem valid and suggest that heparin is not a specific antitryptic agent.

Accordingly, it was thought necessary to reinvestigate the *in vitro* antitryptic action of heparin by means of a standard enzyme method. The Anson-Mirsky hemoglobin digestion method was used. Mixtures of heparin and trypsin were incubated at 25°C. At various intervals a 5 cc fraction of the mixture was added to the hemoglobin substrate and the tryptic activity determined. Mixtures of trypsin with serum were incubated and

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