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The Structure of B. Anthracis and the Reversal of the Gram Reaction.

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If a small amount of aqueous solution of acriviolet (1 oeseful of a 1 per cent solution) be added to ½ cc. of a heavy aqueous suspension of *B. anthracis* it will be found that the organisms are changed by the exposure from sharply Gram positive to sharply Gram negative.* The speed with which this change takes place varies with the strain. In one of the 7 strains examined the change was complete in 2 hours. In other strains it was complete only at the end of 19 hours. Exposure to gentian violet gave results similar to those obtained with acriviolet.

The Gram negative forms present after exposure to these dyes are notably smaller in caliber than the Gram positive forms. Measurements of the stained specimens with a filar micrometer show the loss of caliber to be on the average in the neighborhood of 25 per cent. This measured difference in size in the stained specimens is probably in part due to the piling up of stain on the Gram positive segments since the difference in size, though demonstrable in unstained hanging drop specimens, is of lesser degree than in the stained specimens.

The change in Gram reaction and the change in size cause a striking picture in smears, stained by Gram's method, made from suspensions to which dye has been added, and which are examined at a period when the changes produced are as yet only partial. Chains of bacteria are seen in which stout, deeply Gram positive segments alternate with slender, sharply Gram negative segments. When individual bacteria are present instead of chains, the picture is that of a smear of two different organisms, one stout and Gram positive, the other slender and Gram negative.

A change similar to that produced in *B. anthracis* is also produced by these dyes in most of the other spore bearing aerobes (23 strains examined). The results, however, with some of these strains were not quite so striking nor so constant as in the case of *B. anthracis.* In contrast to the behavior of the spore bearing aerobes the majority

* Burke's modification of the Gram method was used throughout these experiments.

of Gram positive non-spore bearers (notably, the cocci) when exposed to these dyes remain unchanged; they are as sharply Gram positive at the end of the experiment as at the beginning. About fifty strains of Gram positive non-spore bearers were examined and the majority followed the rule just stated. There were, however, exceptions, notably the pneumococci which proved too fragile for this type of experiment and about which no definite statement can be made.

If, therefore, to a mixed suspension containing two sharply Gram positive organisms like *B. anthracis* and *micrococcus freudenreichii* (both of which follow the rule stated above) acriviolet be added and smears, stained by Gram, be examined at the end of 24 hours a striking contrast appears: all, or nearly all, the individuals of *B. anthracis* have become Gram negative while all, or nearly all, the individuals of *M. freudenreichii* remain Gram positive. It is clear from this experiment that the Gram positivity of these two Gram positive organisms is very different in character; in the case of *B. anthracis* it is a surface characteristic rather easily destroyed, the center of the organism being sharply Gram negative.

The phenomena described are due to the facts that *B. anthracis* consists of two distinct parts, a Gram positive cortex and a Gram negative medulla,† and that the cortex may be removed. It may be removed by exposure to acriviolet or gentian violet. It may also be removed, though with much less certainty, by hydrolysis in distilled water. It doubtless sometimes disappears of itself, a fact which accounts for the Gram negative forms (always slender) occasionally seen in smears of *B. anthracis* made from cultures. The existence of a Gram positive cortex and a Gram negative medulla may be beautifully demonstrated by staining smears from a young (4 hr.) culture of *B. anthracis for 5 seconds* with Burke's gentian violet, fixing for *5 seconds* with Burke's iodine, decolorizing for *5 minutes* with acetone ether, and counterstaining with safranin. By this method of rapid staining and intensive decolorization the Gram positive material is partially or completely removed from the cortex and bacteria are seen in all stages of decolorization. They appear either as entirely Gram negative forms, or as sharply Gram negative rods to the surface of which are attached lumps or plaques or dots of undissolved Gram positive material, between which the pink safranin-stained central rod is plainly seen.

That the difference in size between the Gram negative forms re-

† "Medulla" and "cortex" are used tentatively as convenient descriptive terms; "ectoplasm" and "endoplasm" might be better.

sulting from exposure to the dye, and the Gram positive forms in the controls is actually due to a loss of substance seems likely, though perhaps not definitely proven, from the results of experiments in which the bacterial bodies were weighed before and after exposure to the dye. That the material lost is protein, or at least ammoniacal in character, is proven by the positive ninhydrin tests obtained with the Berkefeld filtrate of a suspension of *B. anthracis* which had been exposed to gentian violet.

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Reciprocal Influence of Concomitant Infections: Syphilis and Vaccinia.

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The influence which one infection may have upon the manifestations of another has been investigated in a number of conditions in connection with a general study of the factors concerned in determining the course and outcome of disease processes. In the case of syphilis and vaccinia of the rabbit, the result of simultaneous inoculations of *Tr. pallidum* and vaccine virus has been studied. Other experiments have dealt with the syphilitic reaction in rabbits immune to vaccine virus, and conversely, with the vaccine reaction in rabbits infected with *Tr. pallidum.*

The Nichol's strain of *Tr. pallidum* and the Noguchi strain of vaccine virus were used; groups of 5 or 10 male rabbits were employed for each series. The syphilitic tissue emulsion was injected in one testicle or intracutaneously on the sheath; the inoculation of vaccine virus was made on the shaved skin of the body by rubbing the infected tissue emulsion into scarified areas, and by intracutaneous injection. In other groups, the syphilitic and vaccine virus emulsions were both injected in the same testicle. Control series of rabbits were inoculated with each of these materials.

The observation period varied from 3 to 5 months. An essential requirement of the work was the frequent examination of the rabbits in order that the syphilitic process in the several groups could be compared. The features of this infection which are especially important for such comparisons are illustrated by the following examples: the incubation period, character and duration of the pri-