for the spontaneous occurrence of duodenal or gastric ulcers, but none were found.

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Effect of Hardening and Fixation on Gram Reaction.

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In a recent study of sections of the livers and spleens removed from mice, dead of experimentally produced anthrax, we were surprised to find that although the young culture used to induce the disease had been sharply gram-positive, the majority of the organisms with which the tissues teemed, were either completely or partially decolorized by gram. The experiment was repeated with *Saccharomyces cerevisiae,* a sharply gram-positive organism, more stable in this respect than *B. anthracis.* A heavy suspension of this organism was injected into the anterior abdominal vein of an etherized frog after the other vessels of the liver had been ligated. The liver thus impregnated with yeasts was immediately removed, and pieces of it were put into Zenker's fluid and into formalin. These were run through in the usual manner and cut in paraffine. The sections were stained by gram. (Burke's Modification). A large majority of the organisms were found to be completely or partially decolorized. This reversal of the gram reaction (similar to that recently described as following exposure of *B. anthracis* to acriviolet[1]) might be thought of as occasioned by the contact of the organisms with the animal body, or as resulting from exposure of the organisms to the various chemicals used in hardening and fixation.

An inquiry was therefore instituted as to the effect of these processes on gram reaction. On account of the danger of this kind of experiment if done with *B. anthracis, Saccharomyces cerevisiae*

was used. A heavy suspension of these yeast cells was made and put into two tubes. To one of these Zenker's fluid was added, to the other formalin. At the end of 24 hours, the specimens were centrifugated. The sediment was washed, re-centrifugated and re-washed 10 times during the course of 24 hours. The centrifugate was then run through the alcohols, aniline oil, xylol, and 3 changes of paraffine, just as if sections were to be cut. Smears were made from the last paraffine and were fixed by air drying. The paraffine was removed with xylol, the xylol by alcohol, and the alcohol with water. The smears were stained by Burke's Method. A few entirely gram-positive cells were seen, but the majority were completely decolorized, just as they had been in the sections; that is to say, the processes of hardening and fixation alone had reversed the gram reaction of most of the organisms.

An experiment of another kind was done to test this point. *B. anthracis* was injected into a mouse, which died in 20 hours. Immediately after death, smears were made from liver, spleen and heart's blood. These contained large numbers of *B. anthracis,* practically all of which were sharply gram-positive. Pieces of liver and spleen were then put into Zenker's fluid and run up through the alcohols, aniline oil and xylol. Instead, however, of transferring the material into paraffine and making sections, pieces of it were placed on a slide and crumbled. Alcohol was added to make a suspension, which was smeared and fixed. These smears were again washed with 95% alcohol to remove the xylol, with water to remove the alcohol and stained by Burke's Modification. More than 95% of the organisms were now found to be entirely gram-negative, a few were partially decolorized and only an occasional gram-positive form was seen. It is clear, therefore, that hardening and fixation alone may so alter gram-positive organisms present in tissues that they are no longer capable of retaining the stain. The observation calls attention to a source of error in examining tissue for the presence of gram-positive organisms. It may also account for an idea which prevails, to the effect that the animal body alone is capable of changing the gram reaction of injected organisms. In the experiment last quoted, the organisms in smears from the tissue made just after death, were still sharply gram-positive, and had not been affected in this respect by their residence in the animal body. It was only after they had been through the processes to which tissue to be cut is submitted, that they became gram-negative.

[1] Churchman, J. W., *J. Exp. Med.*, 1927, xlvi, 4007.