LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications

Localization of Bacterial Nucleic Acids and Mechanism of the Gram Reaction

As a consequence of the brilliant studies of Stacey and co-workers, reviewed by Webb[1], many investigators of the Gram reaction in bacteria are agreed that the Gram complex is a combination of basic dye and pentose nucleoprotein, apparently localized at or in the cell wall. However, as Lison[2] has pointed out, the problem of localization in cytochemistry is a difficult one, and it is not altogether certain that the basophilic component of the Gram complex normally occurs at the cell wall.

The site of production of pentose nucleotides has an obvious bearing upon the problem of localization of the Gram complex. From their study of both Gram-positive and Gram-negative bacteria, Malmgren and Hedén[3] concluded that bacteria have two nucleotide-forming systems, one of pentose and the other of deoxypentose type, which form a functional unit but are located in separate organelles within the cell.

Our own studies of fusobacteria, clostridia and lactobacilli indicate that the two structures concerned with nucleotide production form a single morphological unit. But regardless of which view is correct, it becomes evident that the presence of pentose nucleoprotein at the bacterial cell wall represents secondary localization. We have attempted to determine whether this secondary localization is the result of a normal physiological process or an artefact produced by the Gram-staining procedure itself.

Young lactobacilli in smear preparations were stained rapidly with dilute basic dye. Large basophilic polar areas were thus revealed, but there were no basophilic areas adjacent to the cytoplasmic membrane as in the Gram reaction. Gram-stained cells decolorized with acid alcohol and restained with dilute basic dye had diffusely distributed basophilia. When the iodine 'mordant' was omitted in this last experiment, the original distribution of basophilia in simply-stained cells was found. It was concluded, therefore, that the iodine solution is responsible for shifting basophilia from the polar areas to the cell wall.

To test this, a series of preparations were stained with Stirling's gentian violet solution, and then placed in an iodine – potassium iodide solution of *p*H 9·1. Slides were withdrawn at intervals and the Gram staining completed. After 15 min. exposure to the iodine solution, the lactobacilli were Gram-positive, after 30 min. Gram-negative, with Gram-positive polar zones, and after 45 min. again completely Gram-positive. Further treatment failed to effect another reversal of the Gram reaction.

By varying the hydrogen ion concentration, as well as the potassium iodide, it was found possible to influence the speed at which the Gram-negative phase appeared. As the hydrogen ion concentration increased, the time required to reach the Gram-negative phase also increased, while an increase in potassium iodide concentration accelerated the appearance of the Gram-negative phase. It was concluded that there are two basophilic components in lactobacilli which give the Gram reaction; one component is relatively quickly mobilized and can be extracted with iodine solution, whereas the other is only slowly mobilized and cannot be extracted with iodine. Since both basophilic components were hydrolysed by Kunitz-type ribonuclease or $N/1$ hydrochloric acid, it is possible they are pentose nucleoproteins.

The second pentose nucleoprotein component was found as a cortex to nucleal-positive (deoxypentose nucleic acid) polar granules. It differed significantly from the other, since it retained gentian violet staining when the cells were subjected to decolorization without prior iodine treatment.

A less detailed study of *Clostridium septicum* gave results essentially the same as those obtained with lactobacilli.

It is evident that the iodine treatment in the Gram-staining procedure is a complex process involving at least three effects upon various pentose nucleoprotein fractions in the bacterial cell, namely, increased strength of dye binding, alteration of position, and extraction.

The mechanism by which iodine increases dye binding has not yet been elucidated. According to Stearn and Stearn[4], it is due to a greater lowering in the isoelectric point of a protoplasmic component in the Gram-positive organisms than in the Gram-negative. Although no data are on hand to settle the point, a difference in tyrosine content between the pentose nucleoproteins of the Gram-positive and Gram-negative forms could account for such a mechanism, since the iodination of tyrosine increases its acidity[5]. We therefore have reason for suspecting that tyrosine may play an important part in the functioning of cytoplasmic nucleoproteins, and it is of interest to note that Bradfield[6] has postulated that since peptidases do not attack peptides containing phosphotyrosine, it may be possible that combination of tyrosine groups with nucleic acid phosphate might be a means of preventing peptidases from splitting protein during the synthesis of the latter.

This study is from Project No. 323 of the Ohio State University Research Foundation, sponsored by Procter and Gamble of Cincinnati and supervised by Drs. Paul C. Kitchin and Hamilton B. G. Robinson.

Heiner Hoffman

Dental Research Laboratory,
Ohio State University,
Columbus 10, Ohio.

April 20.

[1] Webb, *Research*, **3**, 113 (1950).
[2] Lison, "Histochimie animale" (Paris, 1936).
[3] Malmgren, and Hedén, *Nature*, **159**, 577 (1947).
[4] Stearn and Stearn, *Protoplasma*, **12**, 435 (1931).
[5] Herriott, Adv. Protein Chem., **3**, 169 (1947).
[6] Bradfield, *Exp. Cell Res.*, Supp. 1, 338 (1949).

Alkaline Phosphatase and Contractile Proteins

Alkaline phosphomonoesterase is usually defined as an enzyme with a *p*H optimum around 9·5 which splits monoesters of phosphoric acid. Relatively recently it was found that the comparable acid phosphatase can act as a phosphokinase (Axelrod[1]), and afterwards Meyerhoff[2] showed that alkaline phosphatase also may act as a phosphokinase. The characteristic action of a phosphokinase is the ability to catalyse the transfer of phosphate from one organic molecule to another.