Technical Papers

Reversal of Gram-staining Behavior

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In a recent article, Bartholomew and Mittwer (*1*) report the conversion of gram-positive organisms to gram-negative ones by ultraviolet irradiation. In two recent papers (*2, 3*) we proposed a mechanism for the gram-staining reversal and showed that gram-positive bacteria, as well as alkali-treated wool, could be converted to a gram-negative state by acids or by oxidizing agents and could be reversed back to the gram-positive state by means of alkalis or by reducing agents.

Bartholomew and Mittwer's results appear at first sight to be contrary to the view expressed in our discussion of the possible role of ultraviolet light on the gram-staining reversal (*2*). On further study, however, the results of Bartholomew and Mittwer actually bring added evidence for the mechanism postulated. Meunier (*4*) has shown that the action of light on wool is to make the sulfur more labile and to convert the cystine sulfur to sulfur dioxide and finally to the trioxide, which is freed in the form of sulfuric acid. The experiments of Smith and Harris (*5*) also indicate the formation of sulfate by the photochemical oxidation of wool. Moreover, this was shown to be accelerated by the presence of acids. The formation of hydrogen sulfide appears to be the first step in this process. The protective action of formaldehyde on the degradation of proteins, and of wool in particular, is well known.

It thus seems that the action of reducing agents and of oxidizing agents in the gram-staining mechanism which we postulated can also explain the results of Bartholomew and Mittwer. The action of ultraviolet radiation is probably the following. The first step is a reducing or hydrolytic one whereby —SH and —SOH groups are formed from the cystine linkages. This was the reason for supposing that gram-negative organisms would be converted to gram-positive ones. Mirsky and Anson (*6*) found also that ultraviolet light liberates —SH groups in many proteins. This first step might explain why the formation of gram-negative cells observed by Bartholomew and Mittwer was slow at first and seemed to require an induction period. However, the initial formation of —SH groups is superseded by an oxidation which results in gram-negative response, as we have already postulated. Since formaldehyde has a protective action and a reducing one, its retarding effect on the conversion noted by Bartholomew and Mittwer is readily understood. On the other hand, osmic acid, being oxidizing, would hasten the reaction as observed by Bartholomew and Mittwer. The accelerating effect of acids noted by Smith and Harris (*5*) should be recalled in this connection.

Thus the changes in gram-staining behavior mentioned above emphasize once more the analogy between the behavior of the cytoplasmic membrane of gram-negative bacteria and that of untreated wool, as well as that between gram-positive bacteria and alkali-treated wool (*2*). The role of the ribonucleic acid in the gram stain reversal was also discussed in the above-cited communication.

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Formation of a Labile Pigment in Rabbit Ova During Histochemical Demonstration of Succinic Dehydrogenase[1]

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The histochemical demonstration of succinic dehydrogenase, one of the vital respiratory enzymes, has been previously described (*1–3*). The reaction is based on the reduction of a tetrazolium salt during incubation of fresh tissue in the presence of an excess of sodium succinate (*1*). If neotetrazolium (*pp'*-diphenylene bis 2-(3,5 diphenyl tetrazolium chloride)) (NT) is employed, the reduction of NT is not reversible (*4*). The reduction compound is seen as fine black granules in the cells.

Previously, we demonstrated succinic dehydrogenase activity in ovaries of rabbits injected with urine from pregnant or nonpregnant women (*3*). Fresh 3-mm blocks were incubated for 1 hr at 37° C in 0.9% NT in normal saline with 0.1 *M* phosphate, buffered to pH 7.4 with the addition of 0.03 *M* sodium succinate. The blocks were fixed in formalin neutralized with magnesium carbonate. Twenty-four hr later, frozen sections (15 μ) were cut from the blocks. In the 18 rabbits reported in this series (*3*), as well as in 4 other rabbits separately studied, little or no black pigment

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