8611 C

Effect of Preservatives on Undenatured Bacterial Antigens.*

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In the preparation of undenatured bacterial antigens mass-cultures of recently isolated bacteria are washed free of metabolites and media-constituents with Locke's solution.[1] The cells are fragmented in a special ball-mill[2] and residual intact cells are removed by ultrafiltration.[3] Filtrates containing the chemically unaltered antigenic fractions of the organisms are standardized on the basis of native-protein content and are called Undenatured Bacterial Antigens or more briefly U B A. Antigens of this type have been tested clinically during the past several years.[4-13]

Since the major desideratum of the preparative technic has been avoidance of denaturation it became important to determine just how readily the antigens were altered when exposed to such degrees of heat or chemical action as might be encountered under practical working conditions. We have shown in the case of Staphylococcus U B A[14] that thermal denaturation begins to be significant at 40°C.; the reaction obeys the mass-law and exhibits the remarkably rapid rise in rate with increase in temperature that characterizes protein denaturation in general (critical thermal increment 44,000).

In order to test the adequacy of certain antiseptics to preserve U B A solutions without causing appreciable denaturation, phenol, tricresol and merthiolate were added to aliquots of freshly pre-

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pared staphylococcal U B A in such amounts that the final concentrations were 0.3% tricresol, 0.5% phenol, 0.01%, 0.005%, and 0.002% merthiolate. The solutions were kept at room-temperature for 5 months during which time weekly samples were withdrawn for determination of their content of total, denatured, and native protein. The analytical methods have been described by Krueger and Nichols.[14]

A composite graph of the data obtained in 2 experiments is shown in Fig. 1. It is evident that within the first week at room-temperature the denaturing effect of the preservatives had reached a maximum and after this time equilibrium was established; no further denaturation occurred as long as the temperature was constant. Wide fluctuations from the curves shown in Fig. 1 were observed with a third lot of antigen which was allowed to stand at room-temperature during the summer of 1935. As the temperature rose the concentrations of native protein dropped and those of denatured protein rose. When the room temperature again became 22°C. the content of native protein increased at the expense of the denatured protein and equilibrium again became established at the values recorded in Fig. 1.

Merthiolate in concentrations of 1:50,000 or 1:20,000 produces

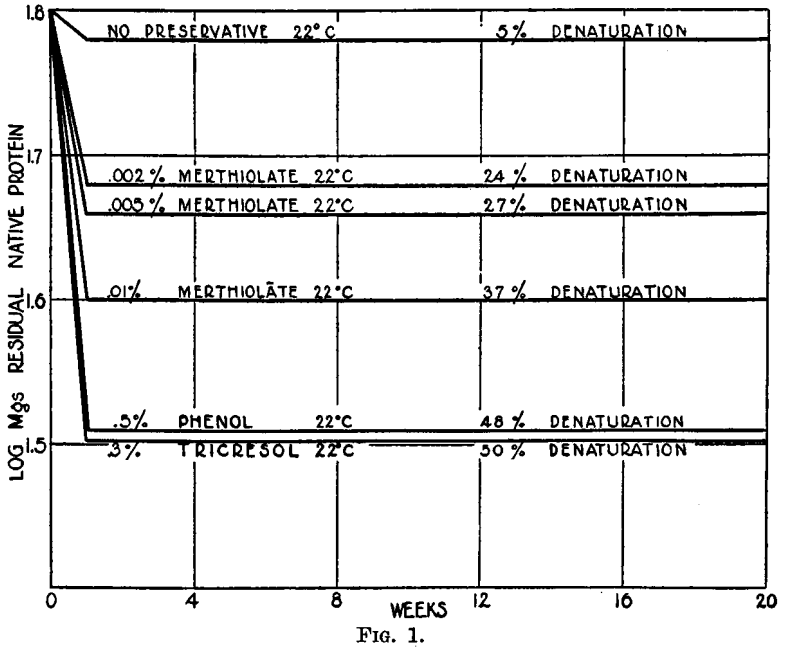

FIG. 1.

The Denaturing Effect of Antiseptics on Staphylococcal Undenatured Antigen.

considerably less denaturation than phenol or tricresol, in the concentrations ordinarily employed for vaccines. It is of practical significance that summer temperatures will shift the equilibrium between native and denatured fractions in favor of the latter, this shift appears to be reversible and the concentration of native protein increases when the temperature is lowered.

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Pure Monophasic Action Potentials and Their Employment in Studies of Ventricular Surface Negativity.

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Recent interpretations of monophasic action potentials led from an injured and uninjured spot on the ventricular surface hold (1) that the entire deflection is dominated by electrical potential alterations at the *uninjured* spot,[1] (2) that it is essentially influenced by potential variations at the *injured* spot,[2] or even more specifically, (3) that the initial rise may be governed by electrical variations at the injured region, whereas its rapid offset is determined by the discontinuance of negativity at the normal region.[3] Further experimentation makes it apparent that correct interpretations have been hampered by registration of impure monophasic waves, *i. e.*, curves that exhibit oscillations or notches either before the rise or somewhere on the ascending limb, or which give evidence of auricular and other extraneous activity. Previous investigators have been obliged to apologize for inescapable extrinsic effects and to devise explanations to account for them. By recording from a discrete spot of injury, as produced by a new type of suction Ag-$AgCl_2$ electrode devised by the author, and another Ag-$AgCl_2$ wick electrode in apposition with the epicardial surface, it proved possible to record consistently monophasic curves that rise acutely from a straight base line and which exhibit no vibrations on the upstroke. An example is displayed in Fig. 1.

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