RECLAMATION OF STREPTOCOCCAL CELLS FOR REUSE IN ADSORPTION OF ANTISERA

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One of the major problems in the production of diagnostic antisera for the specific Griffith types of Lancefield Group A hemolytic streptococci is the accumulation of sufficient volumes of cells for adsorption processes.

To minimize this problem a procedure was developed for the reclamation and reuse of the adsorption cells. The technique is as follows: Packed cells, after use for adsorption, are suspended in 4 to 5 volumes of N/5 HCl in physiological saline and refrigerated at 4 to 10 C overnight. The acid-cell suspension is centrifuged and the cells washed 3 times in 4 to 5 volumes of physiological saline. The second saline suspension is adjusted to pH 7.0 to 7.2 with N/1 NaOH.

Cells treated in the above manner are used routinely for 4 to 5 adsorptions. The maximum number of times they can be so handled has not been determined. However, each of 2 batches of cells has been used for 8 adsorptions with no apparent change in their adsorptive capacity. Untreated cells and cells washed only with physiological saline have not proved satisfactory for reuse.

Resuspension of the tightly packed cells for the acid and saline washes is effected with a midget household electric mixer, the blade of which fits easily into a 50 ml centrifuge tube. Ten to 20 seconds agitation with the mixer yields as even a suspension as 5 to 10 minutes manipulation with a pipette.

There has been no investigation of the efficacy of treatment of the cells with other concentrations of HCl, with other acids, with alkalis, or with high salt concentrations. Although the optimum time of exposure of cells to acid has not been determined, no difference is observed with treatments as short as 4 hours or as long as 4 days. Overnight exposure was adopted arbitrarily as a matter of convenience. No effort has been made to detect antibody in the supernate after acid treatment.

In spite of these voids in information regarding the procedure, it is presented at this time because it has proved practical in routine use. It is hoped that the technique can be adapted for use in the preparation of other bacterial antisera.

THE OCCURRENCE OF "CELL PLATES" IN BACTERIA[1]

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In a recent paper "Cytokinesis in *Gaffkya tetragena*" (Chance, J. Bact., **65**, 593, 1953), structures simulating cell plates were observed in the nucleus and were interpreted as cell plates on the basis of position and behavior during cellular division. The structures were stained differentially by the "crystal violet" nuclear stain of Chance (Stain Technol., **27**, 253, 1952). They were not observed in all nuclei during division and may not be demonstrated during each nuclear division, but yet they were observed clearly in many cases in the above species. At the time, no survey of other species was made to determine the presence of cell plates although they had been

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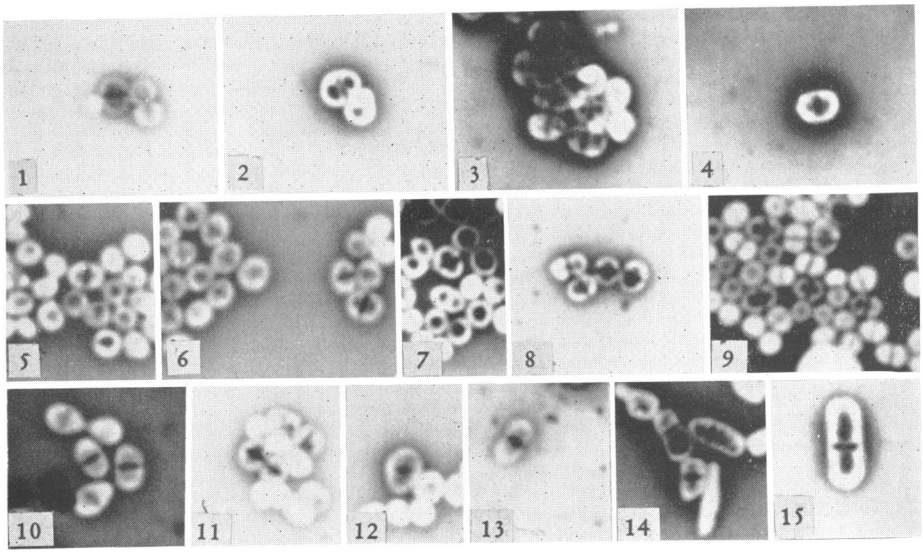

Figures 1–15. Cell plates[1] in various bacteria. The numbers on the plate correspond to the number as given for the organism in the text.

observed in the routine staining of the nucleus of a few species. Since that time, more species, especially of the cocci, have been examined and cell plates observed in the following organisms. 1. *Micrococcus pyogenes* var. *aureus*, 2. *Micrococcus freudenreichii*, 3. *Micrococcus rubens*, 4. *Sarcina lutea*, 5. *Sarcina flava*, 6. *Streptococcus faecalis*, 7. *Streptococcus lactis*, 8. *Streptococcus pyogenes*, 9. *Streptococcus salivarius*, 10. *Neisseria meningitidis*, 11. *Neisseria sicca*, 12. *Corynebacterium pseudodiphtheriticum*, 13. *Serratia marcescens*, 14. *Bacterium mycoides*, and 15. an unknown rod.

It is postulated that these structures will be found in additional species by the method used with *Gaffkya tetragena* and in other species when the proper techniques for their disclosure have been developed.

SELECTIVE INHIBITION OF MICROBIAL GROWTH BY THE INCORPORATION OF TRIPHENYL TETRAZOLIUM CHLORIDE IN CULTURE MEDIA[2]

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In the past decade, extensive use has been made of tetrazolium salts as indicators of dehydrogenases in resting cells (Smith, Science, **113,** 751, 1951). The salts have been incorporated also in bacteriological culture media since the compounds, when reduced by the growing cells, impart color to the developing colonies. This method has been used by Huddleson and Baltzer (Science, **112,** 651, 1950) to facilitate studies of colonial variation and by Goetz and Tsuneshi (J. Am. Water Works Assoc., **43,** 943, 1951) to expedite detection and counting of microcolonies.

Although tetrazolium salts included in media should be nontoxic for the species being studied, toxicity of the compounds for plant and animal

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