NOTES

A SIMPLE MEDIUM FOR THE CULTIVATION OF LEPTOSPIRA

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There are several different types of media used for the cultivation of *Leptospira* species (Stuart, J. Pathol. Bacteriol., **58,** 343, 1946; Chang, J. Infectious Diseases, **81,** 28, 1947; Van Thiel, *The Leptospiroses*, Universitaire pers Leiden, 42, 1948). Some of these are difficult to prepare while others do not maintain good growth over extended periods of time. The medium described in this note is easily made and has proven very satisfactory in maintaining stock cultures and in the preparation of antigens.

This medium is a modification of Gardner's medium (Topley and Wilson, *Principles of Bacteriology and Immunity*, The Williams & Wilkins Co., Vol. I, 920, 1946) and is prepared in the following manner. One capsule of Parstains Buffer Salt Mixture[1] (pH 7.2) is dissolved in 180 ml glass-distilled water. About 15-20 ml of this solution are drawn through a sterile Seitz filter and discarded. Twenty-four ml of fresh rabbit serum[2] (previously tested for antileptospiral qualities) are added to the remaining buffered water together with enough fresh rabbit hemoglobin to give the medium a distinct pink color. The medium is sterilized then by filtering through the prepared Seitz filter and tubed in 5 ml quantities in large screw-capped culture tubes. Immediately after preparation it should be placed in the ordinary type "deep freeze" for storage. Meanwhile about 10 of the tubes may be incubated for sterility.

Seitz filter pads can change the pH of solutions passing through them. It was noted that by filtering a small amount of buffer solution through the pad before using this effect could be reduced until there was no appreciable pH change.

Stock cultures have been maintained for periods up to 3 months without transfer and probably would remain viable for longer periods. This medium will support the growth of small numbers of organisms and has been used as the primary isolation medium when culturing strains of *Leptospira* from blood.

[1] Parstains Buffer Salt Mixture is obtainable directly from Hartman-Leddon Co., Inc., Philadelphia, Pa., and indirectly from scientific supply houses.

[2] To reduce formation of precipitate, each lot should be made with serum from one animal rather than with pooled sera.

A USEFUL BACTERIAL CELL WALL STAIN[1]

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Various techniques for staining the bacterial cell wall have been developed, of which the tannic acid-crystal violet method (Gutstein, Centr. Bakteriol. Parasitenk., **93,** 393, 1924) is one of the most widely used. This technique, which requires a very dilute solution of crystal violet, results in the cell wall and the cross walls of many bacteria being stained rather faintly. The tannic acid-crystal violet technique also has the disadvantage of being prepared as temporary water mounts.

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