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A New Method for Staining Bacterial Capsules.*

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A simple method is described by which a capsule-like structure is readily demonstrated about many bacteria. It is selectively stained pink, in contrast to the blue bacterial bodies. Use is made only of stain and fixative. No artificial steps, like preliminary treatment with serum, etc., designed to render the capsule more readily demonstrable, are taken.

The technic follows: Smear dried in air, covered with 10 drops of Wright's stain, which is left on until it has nearly but not quite evaporated to dryness when a pinkish color replaces the original blue of the stain. This usually takes about 3 or 4 minutes. Wash off as rapidly as possible with Clark and Lub's buffer pH 6.4 to 6.5. (Sometimes a better specimen is obtained if smear is also washed rapidly with distilled water.) Dry with fan without blotting. If washing with buffer is done before stain has evaporated, the capsule-like structures will not be stained. Excellent results may also be obtained by leaving the air dried smear in Wright's stain over night, removing and allowing to evaporate until a pinkish color is reached, then dipping rapidly through buffer and drying with fan.

If technic be carried out correctly the capsule-like structure stains as a pink area surrounding the blue bacteria and itself limited by a definite membranous pink-stained periphery, on the outer surface of which precipitated stain is sometimes observed. Outside this structure a clear zone is occasionally (as in *Diplococcus pneumoniae*) seen. If staining be too deep the whole organism (capsule-like structure and bacterial body) is stained purplish and no distinction can then be made out between the parts. If staining is not deep enough or washing too prolonged all stain is removed from the capsule-like structure, only the blue bacterial bodies being then visible.

Three known capsulated organisms have been studied by this technic [*Diplococcus pneumoniae* types 1, 2, and 3; *B. anthracis* Koch (6 strains); *Klebsiella pneumoniae friedländer* (7 strains)] and the capsule-like structure clearly demonstrated. It occupies the site and has the characteristic of the capsule as demonstrated

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by the usual capsular stains. It is as readily demonstrated on "R" strains of pneumococcus as on "S" strains.

This structure may be made apparently to disappear from *B. anthracis* by suspending the organisms in 1% NaCl and then staining. It reappears however if the organisms are washed free of the salt and restained.

The unfortunate circumlocution "capsule-like structure" has been employed not because the findings are ambiguous but because the capsule has never been accurately defined and the confusion to which the use of terms like "pseudo-capsule", "aureole", "clear zone", etc., has led make it impossible to speak more scientifically. There seems little doubt however that the structure stained by this method is the true capsule.

Two minor defects of this method should be mentioned. Precipitation of stain sometimes produces a granular deposit which makes the background confusing. Experiments now going on indicate that it may prove possible to do away with this disadvantage. Evaporation of Wright's stain also sometimes results in the formation of ringlike bodies which somewhat resemble capsules, although careful study shows them to be artefacts. Though occasionally causing confusion these bodies are usually easily to be distinguished from the true stained capsules.

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Presence of Capsules on "Non-Capsulated" Microorganisms.*

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That capsules may be rather widely distributed throughout the bacterial field has been frequently suggested. Streptococci with capsules were reported by Tavel and Krumbein[1] (*cf.* the *"streptocoque auréolé"* of LeRoy de Barres and Weinberg[2]) and Hiss[3] convinced himself that many if not all strains of *Streptococcus pyogenes* are capsulated. Boni[4] claimed to have demonstrated the presence of

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1 Tavel and Krumbein, *Centralbl. f. Bakt. Ref.*, 1895, **18**, 547.

2 de Barres and Weinberg, *Archiv. de med. et d'anat. pathol.*, 1899, **11**, 399.

3 Hiss, *J. Exp. Med.*, 1905, **6**.

4 Boni, *Centralbl. f. Bakt.*, 1900, **28**, 705.