(3) When salicylic acid is added to the aniline the rate of penetration of cresyl blue into the sap is so greatly reduced that there is no penetration even from cresyl blue solution at pH 9 into the sap at pH 5.5. But the rate of penetration of phenol red is still rapid from the solution at pH 5 into the sap at pH 8.

(4) When oleic acid is added to chloroform, neither cresyl blue nor phenol red penetrates into the sap at any external or internal pH values.

The rate of penetration of dye into the sap is related to the concentration gradient of the dye in the non-aqueous layer. The rapid rate of penetration and accumulation of these dyes is due to the high concentration gradient: these dyes enter the membrane in undissociated form, and are converted by the sap to the dissociated form, which is not very soluble in the membrane.

Determination of the absorption coefficients shows that the reduction in the rate of penetration of dye into the sap in (3) and (4) is due to the increase in the absorption coefficient of the dye at the inner phase boundary which decreases the concentration gradient of the dye in the non-aqueous layer.

Thus the behavior of various cells toward acid and basic dyes can be very roughly imitated by altering the solutions representing the plasma membrane and the sap.

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Bacterial Structure with Particular Reference to the Capsule.

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A technic has been described by which capsules can be readily demonstrated not only on "capsulated" organisms but also on certain bacteria and under certain conditions where they are supposed not to exist.[1] More extensive studies have confirmed the earlier findings, one of the most significant of which was the observation that R types of pneumococcus are as definitely capsulated as S types. Although this method remains the most reliable general capsular stain, with either of the following methods, equally or more beautiful pictures have been obtained, notably with *B. anthracis* from the animal body: modifications of Wright's stain; MacNeal's Tetrachrome Stain; Giemsa Stain; Casares-Gil Flagella Stain. These

[1] Churchman, J. W., and Emelianoff, N. V., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 514.

methods prove that bacterial capsules differ. No one method should be expected to stain all equally satisfactorily. If smears are made from a suspension in 0.25% nutrose, better pictures of the capsule are sometimes obtained.

2. *Structure of Capsule.* The distance between soma and capsular membrane is, like the size of the soma itself, variable. The capsule of virulent pneumococcus enlarges when the organism is injected into the peritoneum of the mouse. The capsule may also shrink and the capsular membrane may then lie practically upon the bacterial body. We have frequently seen 2 distinctly stained capsular membranes, one lying close to the soma and the other at some distance from it, with a large space between. The observations in general suggest that the capsule may in reality be a potential cavity whose size depends on the amount of capsular secretion or other fluid it contains.

3. *Relation of capsule to cortex.* Observations were reported[2] suggesting that many gram positive organisms contain a gram negative medulla and are gram positive only at the surface or cortex. We have recently been able to produce (but not with constancy) bizarre swollen forms of *B. anthracis* in which the medulla (stained pink by Burke's method) can often be seen coursing through the blue black cortex. The relation of capsule to cortex has also now become clear for we have succeeded in staining with differential stains all three layers of *B. anthracis:* the thin pink medullary rod surrounded by the blue black cortex, and quite outside it the eosin stained capsular membrane. Through this capsular membrane chemical interchange must go on freely, for the chemical manoeuvers necessary to bring about partial destruction or decolorization of the cortex show no signs of having affected the capsule in any way.

4. *Relation of capsule to flagella.* If certain motile bacteria are stained by the method of Casares Gil both capsule and flagella are in many instances stained. Usually it is quite clear that flagella arise from the capsular membrane and have no connection whatever with soma. In no instance, when the capsule is stained and the capsular space and membrane accurately located, have we seen the flagella piercing the membrane and passing to the soma. When the flagellum arises from the part of the capsule nearest the observer's eye the appearance may at first suggest origination from soma. In some individuals when the capsular membrane has not taken the stain flagella are clearly seen to originate at some distance from the soma.

[2] Churchman, J. W., *J. Exp. Med.*, 1927, **46**, 1007; *J. Bact.*, 1929, **18**, 413.

Not infrequently when the capsular membrane and space are not demonstrable, the flagella may appear to originate from the somata. Since the capsular membrane may lie practically upon the bacterial bodies and since capsular membrane and soma stain the same color and, if close together, are indistinguishable, it seems not unlikely that in these cases the flagella, though appearing to arise from the bacterial bodies, arise in fact from the capsular membrane itself. The idea that flagella arise from capsule is further borne out by the fact that empty capsules (*i. e.*, devoid of somata) with flagella attached are frequently seen.

5. *Presence of capsule in the acid fast group.* We reported suggestive findings in the study of one member of the acid-fast group *(Mycobacterium tuberculosis bovis)*. The observation was made on specimens stained overnight with carbol fuchsin, decolorized with acid alcohol and then stained by our capsule method. We have since obtained definite evidence of a capsule in this organism by exposing specimens for 3 or 4 minutes to the Casares Gil mordant, washing with water, staining with carbol fuchsin (heated for 3 or 4 minutes, allowed to stand for 10 minutes until cool). The organisms practically always lie in the capsule in an excentric position.

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A Quantitative Study of Adrenal Cortical Hormone Extraction.*

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It was demonstrated[1, 2] that cortical hormone can be prepared from whole beef adrenal glands by essentially the same methods of extraction used in the preparation of cortical hormone from dissected cortex.[3, 4] The elaboration of a biological method of assay based on the minimum maintenance requirement of the adrenalectomized dog[5] provided a reliable means of comparing the potency

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation of New York.

[1] Swingle, W. W., and Pfiffner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 510.

[2] Swingle, W. W., and Pfiffner, J. J., *Am. J. Physiol.*, 1931, **98**, 144.

[3] Pfiffner, J. J., and Swingle, W. W., *Anat. Rec.*, 1929, **44**, 225.

[4] Swingle, W. W., and Pfiffner, J. J., *Am. J. Physiol.*, 1931, **96**, 153, 164, 180.

[5] Harrop, G. A., Pfiffner, J. J., Weinstein, A., Swingle, W. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 449.