possible difference in sulphydryl content between alkali degraded wool and gram positive bacterial membranes on the one hand, and intact wool as well as gram negative bacterial membranes on the other, referred to the cell membrane only and not to the organism as a whole. Since Mittwer and Bartholomew's recent data apply to the whole cell, they have not disproved our inference as to the part played by sulphydryl groups in the gram staining mechanism.

Part of Widra's (J. Bacteriol., 71, 689-702, 1956) work might well serve as an example to illustrate our point. The uni- or bipolar granules of E. coli are revealed to the author "to be lipoprotein and to contain a greater concentration of SH-positive protein than the remainder of the cell"; whereas Bergeren (Widra, 1956) "has provided evidence that the granules in Bacillus megaterium... are associated with the cell membrane."

A METHOD FOR PRESERVING REFERENCE SPECIMENS OF ACTINOMYCETES

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In conducting taxonomic studies with actinomycetes it is frequently desirable to have, for purposes of direct comparison, many reference cultures of different strains or related species. Such material may be required for study over a period of time well in excess of that during which fresh preparations will retain characteristic cultural appearance. Desiccation of the cultures and overgrowth with spreading contaminants are factors limiting the period of usefulness of unpreserved material. The preparation of fresh cultures each time they are needed involves substantial expenditures of time and effort.

Various approaches have been made to the problem of providing permanent or semipermanent reference material useful to the taxonomist. Among these have been photographic techniques which provide natural color reproductions (Dietz, Ann. N. Y. Acad. Sci., 60, 152, 1954); another method involves the preparation of herbarium specimens from dried agar cultures (Jones, Ann. N. Y. Acad. Sci., 60, 124, 1954); a modification of the latter involves growing cultures on filter paper impregnated with nutrient materials, followed by killing and sealing the cultures between two watch glasses (Hessel- tine, NURB, Peoria, Illinois, private commu- nication, 1955). Recently we have employed a simple preservation technique which we find provides reference material that closely approximates fresh, living cultures of actinomycetes.

Our method consists of streaking spores of the organisms on appropriate agar media in petri dishes in a cross-hatched fashion (figure 1) and incubating for 2 weeks at optimum temperatures. One milliliter of 40 per cent formaldehyde per dish is then added dropwise with a pipette to the surface of the agar outside and between the growth zones. The plates are kept at room temperature with covers in place until all of the formaldehyde has been absorbed, usually 2 to 3 hr, after which they are sealed with rubber petri dish sealers (Fisher Scientific Co. Sealer No. 8-759) and stored at 4 C.

Cultures treated in this manner have retained most of their original characteristics for periods longer than 2 years. Also, the plates may be stored at room temperature for several weeks without significant desiccation or cultural changes taking place. Both front and reverse surfaces of the organisms may be readily studied with the dish sealers in place (figure 1), or the seals may be easily removed and the plates opened for microscopic examination when necessary. Morphological features, color of spores or mycelium, and growth habit remain essentially unchanged in most cultures. Occasionally, however, diffusible pigments may gradually fade to duller shades; in rare instances immediate changes in pigmentation have been observed when the formaldehyde was added.

This technique has greatly facilitated our taxonomic studies by making characteristic
specimens of reference cultures always available. Although our experience with it has been largely confined to studies of Streptomyces species, the method has been adapted satisfactorily on several occasions to other groups of microorganisms. Previously, other investigators had successfully prepared tube culture specimens of pathogenic fungi by the addition of formaldehyde to the cotton stoppers (Lewis and Hopper, Arch. Dermatol. and Syphilol., 34, 686, 1936).

OBSERVATIONS ON THE PHYSIOLOGY OF **AZOTOBACTER INDICUM**

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**Azotobacter indicum** has so many properties differing from those of other members of the genus that it has been proposed to place it in a new genus, *Beijerinckia* (Jensen, Bacteriol. Revs., 18, 195, 1954). Among these are: it grows and fixes nitrogen over a wide pH range (3 to 9); it lowers the initial pH to about 3.5 when grown on Burk's or similar nitrogen free media; its growth is slow and accompanied by the production of large quantities of extracellular polysaccharide. In contrast with the intensive studies that have been made on the physiology of *Azotobacter agile*, *Azotobacter vinelandii* and *Azotobacter chroococcum*, few detailed observations on the physiology of *A. indicum* have been published. In connection with an investigation of the composition of the organism's polysaccharide (Quinnell, Knight, and Wilson, Can. J. Microbiol., 3, 277-288, 1957), we have studied some aspects of the organism's physiology particularly with respect to the origin of the long lag period and to the initial drop in the pH. This note presents a summary of our findings; the details of the experiments are available in the Ph.D. thesis of the author—to be published through the dissertation publication service of University Microfilms, Inc., Ann Arbor, Michigan.

The organism was grown in shake flasks, and measurements of growth were made either turbidimetrically or by the determination of cell nitrogen. Volatile acidity of cultures was quantitatively determined by titration of steam distillates, and the acid fractions were identified by paper chromatography. The specific glucose