it might not represent the primary locus of action of the drug, partial reversals obtained with nicotinic acid (30% with 150 μg per ml) and adenine (40% with 20 μg per ml) indicates that purine biosynthesis and thus DPN biosynthesis is inhibited by paludrine.

CULTIVATION OF STAPHYLOCOCCAL L FORMS IN A LIQUID MEDIUM

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Although the L form of other bacterial species has been cultivated successfully in several types of liquid media (Dienes and Weinberger, Bacteriol. Rev., **15,** 245, 1951; Dienes, J. Bacteriol., **66,** 274, 1953; Abrams, J. Bacteriol., **70,** 251, 1955; Altenbern and Landman, J. Bacteriol., **79,** 510, 1960) previous work with the staphylococcal L form had indicated that some concentration of agar was necessary for growth in brain heart infusion broth (Marston, J. Infectious Diseases, **108,** 75–84, 1961).

However, the use of a liquid medium basically similar to that of Altenbern and Landman (J. Bacteriol., **79,** 510, 1960) has permitted the cultivation of L forms of staphylococci in a milieu free of agar. This medium provides abundant growth and should facilitate further study of these L forms.

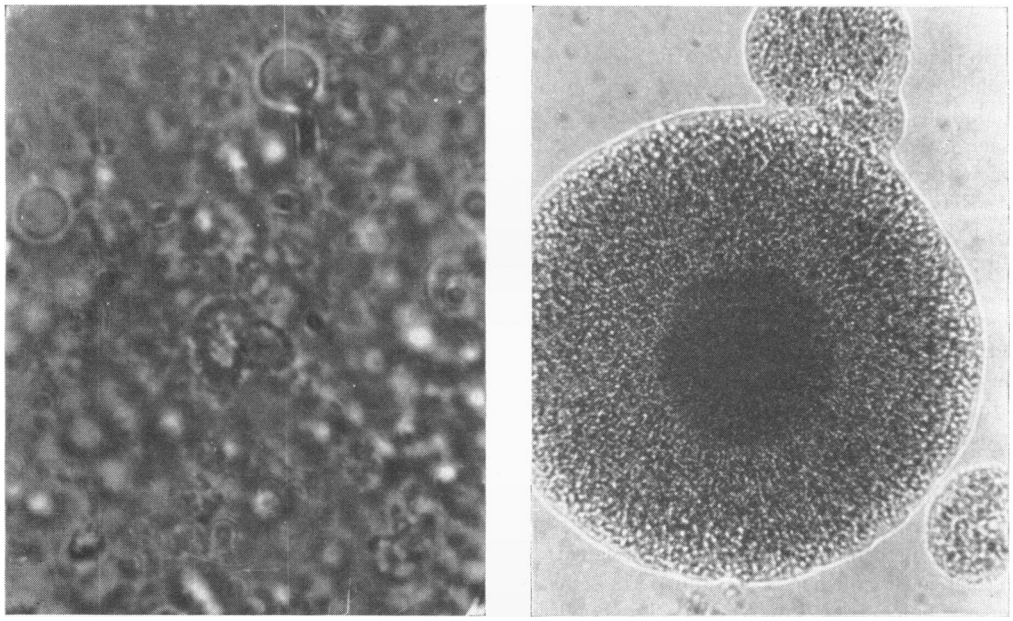

Fig. 1. Granular elements and L colony of staphylococci. *Left,* photograph showing several "large bodies" and indistinct granularity (magnification, 690 ×); *right,* the resultant colony following inoculation of the granular elements onto a suitable agar medium (magnification, 345 ×).

Pure cultures of the L forms of staphylococcus strains SA97, SA96, and phage propagating strain 44A were obtained in a medium of the following composition: Penassay broth (Difco), 17.5 g; NaCl, 46.5 g; distilled water, 1,000 ml. Horse serum to give a final concentration of 10% and penicillin G, final concentration 1,000 units/ml, were added after autoclaving.

[1] From Research Project MR 005.09-1300.1, the Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

This medium, dispensed in 100-ml amounts in 250-ml Erlenmeyer flasks, was inoculated with an agar block containing L colonies which had been growing 5 days following 15 passages on a staphylococcal L form maintenance agar (Marston, J. Infectious Diseases, **108,** 75–84, 1961). Slight turbidity and a granular sediment developed in the liquid medium in 4 days at 37 C and serial transfer of the sediment resulted in more abundant growth in a shorter time.

The sediment was composed of minute, morphologically indistinct elements and some large bodies; no cocci were observed. Many of the large bodies appeared to contain particles which showed Brownian movement. That the granular sediment contained L forms was demonstrated by transferring 0.1 ml to brain heart infusion agar plates with final concentrations of 5% NaCl, 10% horse serum and 1,000 units/ml penicillin G. This transfer resulted in appearance of typical L colonies. Fig. 1 shows the granular elements in liquid media and an L colony derived from them. The colonial morphology was, in all respects, identical to the L colonies used as the initial inoculum.

No reversion of the L forms to cocci was observed following continued cultivation or repeated serial transfer to media free of penicillin and with a lower salt concentration.

Serological and biochemical studies of staphylococcal L forms cultivated in this manner are rendered more tenable and should contribute to the expanding knowledge of them.

MORPHOLOGY OF *NITROSOMONAS EUROPAEA* AND CLASSIFICATION OF THE NITRIFYING BACTERIA

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Interest in the biochemical transformations of the nitrifying bacteria has focused attention on the chemoautotroph *Nitrosomonas europaea.* Descriptions of the morphology of the organism are hampered by difficulties that prevent the application of the usual bacteriological growth techniques to this organism. Until recently, *N. europaea* was grown in calcium or magnesium carbonate-containing media for periods varying from weeks to months. The cells grown under such conditions were often described as nonmotile (Meicklejohn, J. Gen. Microbiol., **21,** 185, 1950), and are listed in *Bergey's Manual of Determinative Bacteriology* (7th ed., The Williams and Wilkins Company, Baltimore, 1957) as nonmotile or with a single polar flagellum, rarely having one at either end. Lewis and Pramer (J. Bacteriol., **76,** 524, 1958) published a picture of *N. europaea* that shows no flagellation.

The organism used in these investigations was kindly supplied by R. L. Starkey and was grown as described by Engel and Alexander (J. Bacteriol., **76,** 217, 1958). When cultures of *N. europaea* were grown for biochemical investigations, the cell levels attained were high enough to prepare electron micrographs. The cells came from cultures having a generation time of approximately 11 hr and growing to a final titer of 2×10^8 viable cells. Cells taken from a late log phase culture were motile and had two subterminal flagella (Fig. 1*a*). The cells were ellipsoidal rods measuring approximately 0.8 by 1.2 μ. Another picture, also showing two flagella, appears elsewhere (Engel, Ph.D. thesis, Cornell University, 1959).

The difficulties in showing flagella in an electron micrograph were as follows: Centrifugation caused rupture and produced a flagella-free population (Fig. 1*b*). This rupturing could also explain the absence of flagella in the pictures of Lewis and Pramer (*personal communication*) and Hofman and Lees (Biochem. J., **53,** v., 1953). When the population reached a titer of 2×10^8 cells, the nitrite-nitrogen level in the medium was more than 1 mg/ml. This nitrite level causes corrosion of the copper grids on which the bac-

[1] Work performed at Cornell University, Ithaca, New York.

[2] Operated by Union Carbide Corporation for the U. S. Energy Commission.