cent of incorporation continues to occur, whereas in intact cells the amount of the residual protein synthesis under CM inhibition is about 5 per cent. Since incorporation of amino-acid in this system is not inhibited by penicillin (40 μg per ml.), this may not be related to synthesis of cell wall substance. The nature of this residual incorporation remains to be ascertained.

Our results conflict with Ramsey's observation on CM resistant cells of *Staph. aureus*. Two possibilities may be considered. First, in the CM resistant strain of *Staph. aureus*, there may be a CM resistant pathway of protein synthesis which is not present in *E. coli*. Secondly, in the disrupted cell system used by Ramsey, some sort of permeability barrier may be still present.

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[1] Kuschner, D. J., *Arch. Biochim. Biophys.*, **58**, 347 (1955).
[2] Ramsey, H. H., *Nature*, **182**, 602 (1958).
[3] Yokota, T., and Akiba, T., *Medicine and Biol.* (Tokyo), **58**, 172 (1961).
[4] Lamborg, M. R., and Zamecnik, P. C., *Biochem. Biophys. Acta*, **42**, 206 (1960).
[5] Matsubara, K., and Watanabe, I., *Biochem. Biophys. Res. Comm.*, **5**, 22 (1961).
[6] Hirokawa, H., Abe, M., Mizuno, D., *Jap. J. Med. Sci. and Biol.* **12**, 119 (1959).

A Selective-Diagnostic Medium for the Isolation of Pectinolytic Organisms in the Enterobacteriaceae

In the family Enterobacteriaceae, the genus *Erwinia* has been divided into those which cause dry necroses, galls or wilts in plants, and those which produce the enzyme protopectinase and cause soft-rots in plants[1]. The latter group has been given the name *Pectobacterium* by Waldee[2].

The ability to liquefy pectate gels is of great importance in characterizing the soft-rot *Erwiniae* (Pectobacteria), and the technique devised by Paton[3] for preparing pectate gel has simplified the use of the test.

The isolation of soft-rot *Erwiniae* where large numbers of other bacteria (*Escherichia*, *Aerobacter*, *Pseudomonas*, *Bacillus*, etc.) are present may be somewhat difficult, and necessitate the examination of many types of colonies from primary plate media. The salicin medium of Noble and Graham[4] relies on the inhibiting action of a bile salt (sodium taurocholate) to prevent the growth of organisms other than those in the Enterobacteriaceae. The fermentation of the salicin is used as a diagnostic characteristic for recognizing possible soft-rot erwiniae. Colonies are slow to develop on this medium, which has only an ammonium salt as a nitrogen source.

It was felt that a solid plating medium combining the inhibitory power of bile salts and the diagnostic characteristic of pectate liquefaction, along with lactose or salicin fermentation, would prove of some value for isolating soft-rot *Erwiniae* from material heavily contaminated with other types of bacteria.

The following medium was devised:

Basal layer	
McConkey agar granules ('Oxoid *C.M.*7')	5·2 g
Calcium chloride	0·4 g

These were dissolved with boiling in 75 ml. distilled water. The medium was autoclaved at 10 lb. pressure for 10 min, cooled and pipetted in 15 ml. amounts into sterile Petri plates.

Pectate layer	
Sodium polypectate ('Exchange' brand)	2 g

This was suspended in 6 ml. of ethanol. 0·1 g of ethylenediamine tetraacetic acid (disodium salt) was dissolved in 100 ml. of distilled water, and this solution was added to the pectate suspension to a final volume of 100 ml. This medium was adjusted to *p*H 7·4, autoclaved at 10 lb. pressure for 10 min, and after cooling to about 55° C, was pipetted in 5-ml. amounts on to the cold, gelled McConkey agar base in the Petri plates.

The pectate layer set in a few minutes, and was ready for streaking after overnight drying at 37° C.

After 48 h incubation at 25° C, *Erwinia*, fermenting lactose, develops as red colonies in shallow pits formed from the liquefaction of the pectate. *Escherichia*, *Aerobacter* and other lactose fermenting, non-pectate liquefying bacteria appear as red colonies with no surrounding pit in the medium. Non-lactose fermenting bacteria produce yellow or orange colonies.

Salicin could be used instead of lactose in the basal layer where the type of *Erwinia* and/or the types of contaminating bacteria indicated its use. Its use would, however, involve the preparation of McConkey agar from its basic constituents.

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[1] *Bergey's Manual of Determinative Bacteriology*, Seventh Ed., 349 (1958).
[2] Waldee, E. L., *Iowa State Coll. J. Sci.*, **19**, 435 (1945).
[3] Paton, A. M., *Nature*, **183**, 1812 (1959).
[4] Noble, M. and Graham, D. C., *Nature*, **178**, 1479 (1956).

CYTOLOGY

An Electron Microscopic Demonstration of a Surface Pattern on the Plasma Membrane of Sectioned Intestinal Epithelium after Saponin Treatment

A transverse section of the plasma membrane, after osmium or permanganate fixation, appears in electron micrographs as a triple-layered structure, about 8 mμ thick, composed of two outer opaque layers separated by a clear layer[1]. This unit membrane appears to be universal for all higher animal and plant cells and has been considered to represent the protein-lipid-protein molecular model as originally proposed for the plasma membrane by Danielli and Davson[2]. Until the recent report by Dourmashkin, Dougherty and Harris[3], little electron microscopic evidence had been produced to suggest heterogeneity on the plane surface of the plasma membrane. These authors demonstrated, however, that *in vitro* saponin treatment produced hexagonally arranged pits or holes in isolated membranes from erythrocytes, liver cells, Rous sarcoma cells and virus particles. The pits were seen in isolated membranes spread on grids after negative staining, and they