NOTES

A SOLID MEDIUM FOR DETECTING COLONIAL VARIANTS OF *PASTEURELLA PESTIS*¹

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A major problem in detecting colonial variants is the development of a suitable solid medium that will permit their positive identification. Levine and Garber (J. Bact., **60**, 508, 1950) incorporated tetrazolium chloride (TPTZ) in a tryptose agar medium that satisfactorily distinguished smooth and rough colonies of *Pasteurella pestis*, primarily on the basis of differences in color. Since a transparent medium illuminated by obliquely transmitted light had been successful in studies on the dissociation of *Brucella* (Braun: J. Bact., **51**, 327, 1946), a similar medium and a similar technique for observing colonies of *P. pestis* were considered. A comparable medium, designated as TGY, was developed and proved superior to the TPTZ medium in ease of preparation and in stability during storage. The TGY medium has the following composition: 2 per cent tryptone (Difco), 0.2 per cent glucose, 0.8 per cent yeast extract (Difco), and 2 per cent agar. To ensure relatively uniform thickness, approximately 20 ml of medium were dispensed in petri dishes.

Colonies, on streaked plates of TGY medium, grown for 48 hours at 30 C were observed by Braun's method, using a light blue filter in the light source. Typical smooth colonies were cream white and opaque with a smooth, glistening, convex surface and a regular margin, whereas rough colonies were light blue and translucent with a rough, deeply furrowed, flat surface and an irregular margin. Satisfactory differentiation of colonial types was demonstrated with TGY medium that had been stored in the refrigerator for 14 days prior to inoculation by streaking from a liquid culture.

Colonies that could not be classified as typically smooth or rough were found to maintain their morphological characteristics when subcultured on TGY medium. A rough colony was isolated from a highly virulent, smooth strain of *P. pestis*. Subsequent transfer on TGY medium demonstrated the stability of this rough variant. Subcutaneous inoculations in a susceptible host, the mouse, revealed the avirulence of this rough variant.

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