NOTES

USE OF CRYSTAL VIOLET OR BRILLIANT GREEN DYES FOR THE DETERMINATION OF SALMONELLAE IN DRIED FOOD PRODUCTS

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The greater efficiency in the recovery of small numbers of salmonellae from dried foods, particularly dried egg albumen, by enrichment of the food in lactose broth as contrasted to direct inoculation of selective enrichment broths has been shown (*unpublished data*).

Another type of enrichment has been found applicable for the examination of other desiccated foods such as dried milk, baby foods, etc. Experiments have demonstrated that 0.002 per cent brilliant green or 0.004 per cent crystal violet in reconstituted nonfat dried milk will permit unrestricted growth of salmonellae and coliform bacteria when small numbers of the organisms are artificially introduced. At the same time the growth of *Streptococcus lactis* is inhibited, as shown by the lack of significant lowering of the pH for 48 hr or more, depending upon the numbers of cells of *S. lactis* added.

Sterile reconstituted nonfat dried milk (100 g/liter) with 0.04 per cent brilliant green and artificially inoculated with *Salmonella tennessee* in low numbers (approximately 1/ml) was incubated for 24 hr and streaked on brilliant green agar. Large numbers of salmonellae were observed on the plates. Higher concentrations of the dye (up to 0.07 per cent) restricted growth for 24 hr but not for 48 hr. No multiplication occurred in milk with 0.1 per cent dye even when incubated for 72 hr.

At the recommended concentration of 0.002 per cent brilliant green or 0.004 per cent crystal violet there is negligible inhibition of salmonellae and small numbers can be detected. But, direct streaking on brilliant green agar should be supplemented by loop inoculation into selenite-cystine broth (North and Bartram, Appl. Microbiol., **1,** 130, 1953) which is incubated 6 to 24 hr before streaking on selective agar media.

Dried egg albumen has been examined for salmonellae by enrichment in sterile reconstituted nonfat dried milk with dyes at these concentrations. After incubation periods of 24, 48, and 72 hr and at much longer periods up to 8 days, excellent recovery was obtained without "skips" in the most probable number determinations both by direct streak, and by loop inoculation into selenite-cystine enrichment broth. The longer enrichment periods, up to 72 hr, may be necessary for detection of salmonellae in the highest dilutions.

DIFFERENTIATION OF VARIOLA FROM OTHER MEMBERS OF THE POXVIRUS GROUP BY THE PLAQUE TECHNIQUE

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Several investigators (Noyes, Proc. Soc. Exptl. Biol. Med., **83,** 426, 1953; Youngner, J. Immunol., **76,** 288, 1956; Porterfield and Allison, Virology, **10,** 233, 1960) report that vaccinia virus and other poxviruses are capable of forming plaques on monolayers of susceptible tissue cells. This note describes a method of differentiating variola and alastrim from other mem-