

cose yeast-extract agar. Eleven colonies were selected for further study. These cultures were still resistant after storage for nine months.

Flasks containing glucose yeast-extract calcium-carbonate medium were inoculated with 1 per cent of a 24-hour inoculum of these streptothricin-resistant cultures and also from the streptothricin-susceptible parent culture. These flasks were incubated at 30 C for eight days and were analyzed at the end of that interval for glucose, acetoin, ethyl alcohol, and 2,3-butylene glycol. Over 98 per cent of the glucose (Shaffer and Somogyi method, J. Biol. Chem. **100**, 695) was fermented in all cases (correction being made for acetoin present). 2,3-butylene glycol was determined by the method of Johnson (unpublished). A modification of the Langlykke and Peterson (Ind. Eng. Chem. Anal. Ed. **9**, 163) method for the determination of acetoin was used. Ethyl alcohol was determined by dichromate oxidation of the neutral solvents' distillate (and corrections made for acetoin and 2,3-butylene glycol present). Results are summarized in table 1.

The variation in the solvents' yields is about that found in between fermentations by nonresistant (untreated) isolations from the parent culture. This same variation is given by isolations from isolate #1 of table 1. It may be concluded that the fermentations of streptothricin-resistant cultures are not significantly different from those of the streptothricin-susceptible parent cultures.

## THE GROWTH FACTOR REQUIREMENTS OF CERTAIN STREPTOCOCCI

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Studies on the nutritive requirements of the streptococci have shown that, although certain of them may be grown successfully in a synthetic medium, others require the presence of additional natural materials for growth (Wooley and Hutchings, 1939, 1940; Woolley, 1941; Bass, Berkman and Saunders, 1941; Grossowicz, 1942; Niven, 1943). Through the courtesy of Dr. Smith we have been supplied with cultures of *Streptococcus lactis* which failed completely to grow in a synthetic medium and appeared to require the presence of a new growth factor (Smith, 1943). In addition we have studied the nature of a closely related factor or factors highly stimulative for a strain of *Streptococcus fecalis*.

In agreement with the recent report of Niven (1944), we had found that asparagine was effective in promoting growth of *Streptococcus lactis*. Under our conditions, purified glutamine was ineffective in lieu of asparagine. Neither asparagine nor glutamine, nor a combination of the two, was effective in stim-

lating the growth of the strain of *Streptococcus fecalis*. We have found, however, that in the absence of asparagine and glutamine, trypsinized *vitamin-free* casein is effective in promoting growth of both *Streptococcus lactis* and *Streptococcus fecalis*. In the case of *Streptococcus lactis*, the activity of the casein digests in promoting growth is much greater than can be accounted for on the basis of the aspartic (or glutamic) acid content of casein. Obviously a factor or factors more highly active than asparagine (or glutamine) must be present. It is suggested that asparagine or glutamine or both may suffice as growth factors for certain streptococci but that they are involved in the structure or synthesis of more highly active compounds which function in the nutrition of the more fastidious strains.

## REFERENCES

- BASS, A., BERKMAN, S., AND SAUNDERS, F. 1941 An additional growth factor needed by some hemolytic streptococci. *J. Infectious Diseases*, **68**, 220-225.
- GROSSOWICZ, N. 1942 A new growth factor for hemolytic streptococci. *Proc. Soc. Exptl. Biol. Med.*, **49**, 8-11.
- NIVEN, C. F. 1943 The nutrition of group B streptococci. *J. Bact.*, **46**, 573.
- NIVEN, C. F. 1944 Nutrition of *Streptococcus lactis*. *J. Bact.*, **47**, 343-350.
- SMITH, F. R. 1943 Nutritional studies on *Streptococcus lactis*. I. An unidentified growth factor found in yeast extract. *J. Bact.*, **46**, 369-371.
- WOOLLEY, D. W., 1941 A new growth factor required by certain hemolytic streptococci. *J. Exptl. Med.*, **73**, 487-492.
- WOOLLEY, D. W., AND HUTCHINGS, B. L. 1939 Some growth factors for hemolytic streptococci. *J. Bact.*, **38**, 285-292.
- WOOLLEY, D. W., AND HUTCHINGS, B. L. 1940 Synthetic media for culture of certain hemolytic streptococci. *J. Bact.*, **39**, 287-296.