

An Enrichment Broth for Isolating Hemolytic Streptococci from Throat Swabs.

ROBERT M. PIKE. (Introduced by S. Edward Sulkin.)

From the Department of Bacteriology, Southwestern Medical College, Dallas, Texas.

A number of procedures have been described to assist in the isolation of streptococci from mixed cultures. These include methods directed towards the elimination of *Hemophilus hemolyticus*,^{1,2} the use of crystal violet to inhibit staphylococci,³⁻⁶ and the use of potassium tellurite^{4,7} or sodium azide⁶⁻¹⁰ to inhibit Gram-negative bacteria. We have observed that hemolytic streptococci in blood broth tend to outgrow other streptococci and *Neisseria* found in throat cultures. Taking advantage of this fact as well as the selective inhibitory properties of crystal violet and sodium azide, it has been possible to increase the number of hemolytic streptococcus isolations from throat swabs. Without the inhibitors the rapid growth of a few staphylococci or coliform bacilli present on the swabs usually obscured the hemolytic streptococci completely.

Beef heart infusion agar plates containing 5% rabbit blood were inoculated directly with throat swabs. The inoculum was spread with a loop to obtain isolated colonies. The same swabs were then placed in tubes of blood infusion broth prepared as follows. Beef heart infusion broth containing 1% tryptose, 0.02% glucose, and 5% rabbit blood was

tubed in 2 ml amounts. On the day the medium was used, to each tube was added 0.15 ml of an autoclaved aqueous solution of sodium azide, 1:1000, and 0.1 ml of an autoclaved aqueous solution of crystal violet, 1:25,000. Experiments showed that the broth began to lose its growth-inhibiting properties if allowed to stand for 2 days after the addition of the sodium azide. Swabs were cultured within one hour after being taken. After incubation overnight, subcultures were made on blood agar plates by streaking from the broth with a straight needle. Hemolytic streptococci were isolated and Lancefield serological groups were determined with formamide extracts.

Results. Staphylococci, *Neisseria*, *Hemophilus*, and coliforms were largely inhibited in the broth cultures. Although alpha and gamma streptococci appeared in large numbers on most of the plates streaked from broth, the isolation of beta hemolytic streptococci was greatly facilitated.

Table I summarizes the results with 131 throat swabs* from well children taken during June and the first week of July. The total number of positive cultures was increased from 16 to 51 by the use of broth; the number positive for Group A from 6 to 26, and for Group C from 8 to 17. The increase in total positive cultures and in those positive for Group A is statistically significant. The number of hemolytic streptococcus colonies on each plate sufficiently separated to be fished directly was noted. The proportion of positive plates with 5 or more such colonies was only 24% on direct plating as compared with 60% for plates streaked from broth. Also, hemolytic streptococci predominated on 23% of the positive plates from broth, while none of the direct plates showed a preponderance

¹ Mueller, J. H., and Whitman, L., *J. Bact.*, 1931, **21**, 219.

² Krumwiede, E., and Kuttner, A. G., *J. Exp. Med.*, 1938, **67**, 429.

³ Edwards, S. J., *J. Comp. Path. Therap.*, 1933, **46**, 211.

⁴ Rose, K. D., and Georgi, C. E., *Proc. Soc. Exp. Biol. and Med.*, 1941, **47**, 344.

⁵ Fleming, A., *Brit. Med. J.*, 1942, **1**, 547.

⁶ Stiles, M. H., and Chapman, G. H., *J. Bact.*, 1942, **43**, 64.

⁷ Fleming, A., *J. Path. Bact.*, 1932, **35**, 831.

⁸ Snyder, M. L., and Lichstein, H., *J. Inf. Dis.*, 1940, **67**, 113.

⁹ Lichstein, H., *J. Bact.*, 1941, **42**, 293.

¹⁰ Packer, R. A., *J. Bact.*, 1943, **46**, 343.

* The author is indebted to Dr. Gladys Fashena, Department of Pediatrics, for this material.

TABLE I.
Isolation of Hemolytic Streptococci in 131 Throat Cultures.

	Plates inoculated directly from swabs	Plates streaked from broth cultures
Total positive cultures	16 (12.2%)	51 (38.9%)
Group A	6 (4.6%)	26 (19.8%)
" C	8	17
" F	0	2
" G	3	4
Not grouped	0	2

of hemolytic streptococci. In one instance both Group A and Group C organisms were isolated from the direct plate but only the C was recognized on the plate from broth. From another swab, the direct plate yielded Group A and the plate from broth, Group C.

The Group A carrier rate of 19.8% in this limited series is high as compared with that

recorded by other investigators.¹¹⁻¹⁴ Further studies, now in progress, may indicate an even higher rate in winter months.

Summary. By culturing 131 throat swabs from normal children in a partially selective broth prior to plating, the observed hemolytic streptococcus carrier rate was tripled. Group A streptococci were isolated from 19.8% of the swabs.

¹¹ Hare, R., *Lancet*, 1940, **1**, 109.

¹² MacDonald, I., Simmons, R. T., and Keogh, E. V., *Med. J. Australia*, 1940, **1**, 849.

¹³ Rantz, L. A., *J. Inf. Dis.*, 1941, **60**, 248.

¹⁴ Schwentker, F. F., Janney, J. H., and Gordon, J. E., *Am. J. Hyg.*, 1943, **38**, 27.

14748

Sesame Protein in Chick Diets.

C. R. GRAU AND H. J. ALMQUIST.

From the Division of Poultry Husbandry, University of California, Berkeley.

In a previous note¹ the mutual supplementary action of soybean meal and sesame meal was reported, together with the results of preliminary experiments with sesame protein as the only source of protein in the diet of the chick.

The first experiment of the present series (Table I) was carried out concurrently with the last experiment in the previous report. To the basal diet in which sesame meal was the only protein source, 10% of casein, gelatin, or zein was added. After the chicks had been receiving the diets for 4 days, it appeared that the zein was ineffective in increasing the growth rate over that observed on the basal diet, while casein promoted optimal and gelatin slightly suboptimal growth. From these

results and from recent data on the amino acid content of casein,² gelatin,² and zein,³ a lysine deficiency in sesame meal was suspected (Table I). On the fourth day of the experiment, *l*(+)-lysine monohydrochloride was added to the zein-supplemented diet to provide 0.9% of the free amino acid. After 24 hours, the chicks in this group had gained enough to make their growth curve parallel to that of the casein group, and they gained at this rate for 4 more days. When the chicks were then returned to the unsupplemented diet, growth practically ceased, as in the beginning of the experiment. When 0.45% lysine was added after 5 more days, the growth

² Block, R. J., and Bolling, D., *J. Am. Dietetic Assn.*, 1944, **20**, 69.

³ Vickery, H. B., *Compt. Rend. trav. lab. Carlsberg, Ser. chim.*, 1938, **22**, 519.

¹ Almquist, H. J., and Grau, C. R., *Poultry Sci.*, 1944, **23**, 341.