i.e., pH 7.3 (N-Z Amine YT), and pH 7.2 (N-Z Amine YT + NaCl).

Growth stimulation by NaCl was noted (Fig. 1) in both plain and sugar broths, although the development of acid apparently limited growth in the latter media. Glucose utilization and acid production (Fig. 2) were also increased by NaCl, generally in parallel with the stimulation of growth. A slight drop in pH in the glucose-free media during the first 5-hr period was noted (0.28 pH units in N-Z Amine YT and 0.32 pH units in N-Z Amine YT-NaCl broths), which seems to have partially accounted for the initial rapid rise in titratable acidity in glucose media (Fig. 2). Since glucose in the basal medium was below measurable quantities, the initial acidity was apparently due to utilization of other substances. In other experiments, NaCl had a greater stimulating effect when added aseptically from concentrated solutions after sterilization than when incorporated before autoclaving. When NaCl was added aseptically, optimal growth was noted at salt concentrations of 1.0% in 0.5% glucose broth, but was less definite in glucose-free broth ranging from about 1.0 to 2.0%. Although markedly reduced, growth was observed in salt concentrations as high as 8.0% in both glucose-containing and glucose-free media.

Growth stimulation may be a factor in the stimulation of swarming of *Proteus*, which has been reported with NaCl concentrations of 0.5% (Naylor, J. Med. Lab. Technol. **17**:1, 1960; Schmeierson, J. Bacteriol. **82**:621, 1961) and reported to be optimal at 0.5 to 1.0% NaCl after 24 hr and at 2 to 3% after 48 to 72 hr (Kopper, J. Bacteriol. **84**:1119, 1962). NaCl also may act by widening the optimal range of growth under certain environmental conditions, as has been observed with *Escherichia coli* at various pH ranges (Holm and Sherman, J. Bacteriol. **6**:511, 1921). The latter is suggested by the higher levels of acid production and glucose utilization observed in cultures of *P. vulgaris* at lower pH levels in the presence of added NaCl, even though the organism grew better under alkaline conditions. The results suggest the possible value of studying further the effect of NaCl on growth and metabolism of *P. vulgaris* in chemically defined media.

IMPROVED CAT TEST FOR ENTEROTOXIN

C. B. DENNY AND C. W. BOHRER

National Canners Association Research Foundation, Washington Research Laboratory, Washington, D.C.

Received for publication 8 April 1963

The intraperitoneal injection into cats of heated growth material of *Staphylococcus aureus* strains and of food slurries suspected of containing staphylococcal enterotoxin has been used by some workers (Matheson and Thatcher, Can. J. Microbiol. **1**:372, 1955) in the public health field and has been considered uncertain by others (Hussemann and Tanner, Food Res. **14**:91, 1949). Nonspecific emetic reactions have been indicated in the literature (Dolman, Can. J. Public Health, **34**:97, 1943).

The test, conducted by the injection of 2 ml of growth material of *S. aureus* (heated for 30 min at 212 F to destroy hemolysins), has given positive results in well over 50 tests in this laboratory. Control tests (42) with a Casamino Acids medium alone have given no false positive reactions, nor have 11 control inoculations of Brain Heart Infusion medium.

However, the liquid from *Escherichia coli* (ATCC 11303) grown in sterile ham and heated for 30 min at 212 F has given two positive reactions out of three cats tested. This culture grown in Brain Heart Infusion and heated for 30 min at 212 F has given four positive reactions out of six cats tested. Also, a culture from ham (no. 5) that has the characteristics of a *Proteus* strain gave the same ratio of positive reactions after growing in these media and being heated for 30 min at 212 F.

At this point, the results obviously indicate that the cat test performed by injecting growth material heated for 30 min at 212 F intraperitoneally, and observing for an emetic reaction,

in cats is not a specific test for enterotoxin. This would mean that the test can be used only in testing for enterotoxin from a pure culture of *S. aureus*.

The literature indicates that enterotoxin can be concentrated within a dialysis sac (Thatcher et al., Can. J. Microbiol. **1**:401, 1955). Dialysis has been used in the preliminary steps in concentrating enterotoxin from food (Davison and Dack, Food Res. **7**:80, 1942). Normally, the enterotoxin will not pass the dialysis sac; however, in unpublished data from Clampit et al. (reported in Davidson, *Studies on Staphylococcus Enterotoxin*, University of Chicago Press, 1940) enterotoxin has been indicated to pass through a collodion membrane (3.5-mμ pore size) under pressure, but no details are given. An easy means of pulling a vacuum on dialysis tubing has recently been found; it utilizes dialysis tubing stretched over the nylon framework of an LKB 6300 A Ultrafilter (LKB Produkter AB, Stockholm, Sweden, Fisher Scientific Catalogue No. 9-743). Ultrafiltration is said to eliminate large molecules of substances such as proteins and bacteria. Solutes with a molecular weight of less than about 30,000 are allowed to pass (according to the manufacturer) and this should allow enterotoxin (approximately 23,000) to pass. With dialysis tubing with an average pore radius of 24 A and a vacuum of only 12 in. in an LKB Ultrafilter, *Staphylococcus* 196-E (type A) enterotoxin passed through the sac. With this treatment, six positive reactions out of six cats tested have resulted. The possibilities for the use of the ultrafilter for testing suspected food is apparent.

However, the substance emetic in cats produced by *E. coli* and the *Proteus* (no. 5) also passed the ultrafilter at 12 in. of vacuum.

One means of freeing enterotoxin from its hemolysins is to digest the growth material with trypsin (E. P. Casman, *personal communication*). This procedure has worked well in this laboratory, although the problem of getting rid of the trypsin remains. It was found in tests in our laboratory that the ultrafilter effectively removed the trypsin at 12 in. of vacuum. No trypsin could then be detected by the safranin test (Effront and Prescott, *Biochemical Catalysts in Life and Industry*, p. 303, 1917, John Wiley & Sons, Inc.). The cats gave emetic reactions and died when injected with ultrafiltered enterotoxin, but gave only the emetic reaction and lived when injected with unheated, digested, ultrafiltered enterotoxin, indicating that the digestion effectively removed hemolysins. Also, the toxin appeared to be even more potent if it was first digested with trypsin and then ultrafiltered.

It was found that the emetic factor produced by *E. coli* and the *Proteus* strain also survived digestion and ultrafiltering under vacuum. In addition, these emetic factors also survived digestion followed by heating for 30 min at 212 F. However, only the enterotoxin has given an emetic reaction after the combination treatment in this order: digestion, ultrafiltering, and heating.

An effective method of separating staphylococcal enterotoxin from the emetic factors of *E. coli* and *Proteus* (no. 5) was found to be as follows: (i) add trypsin to the growth material to give a 1% concentration; (ii) digest for 1 to 2 hr at 98 F; (iii) cool rapidly, centrifuge, and save the supernatant fluid; (iv) ultrafilter the supernatant fluid at 12 in. of vacuum (use of higher vacuum fails to separate the *Proteus* emetic factor from enterotoxin) through dialysis tubing (pore radius, 24 A); (v) heat the material from inside the ultrafilter for 30 min at 212 F; (vi) inject 2 ml of the material intraperitoneally into a healthy cat and observe for 2 hr (the test is conducted about 2 hr after feeding); (vii) a positive emesis of food indicates staphylococcal enterotoxin.

The use of the ultrafilter in concentrating and purifying enterotoxin preparations is suggested.

This investigation was supported in part by U.S. Public Health Service research grant EF-124(C1) from the Environmental Engineering and Food Protection Division. The authors are indebted to M. S. Bergdoll for the culture of 196-E and to E. P. Casman for suggestions on the media used.