

color from ammonia was produced by an additional 5 min of incubation at 37 C. All strains of *Proteus*, except those of *P. inconstans*, generated detectable amounts of ammonia (Table 1). None of the *Escherichia* organisms was urease-positive. These findings were identical with those yielded by the urease technique of Stuart et al.

The sensitivity of the Berthelot color reaction permits detection of urease activity of single-colony isolates within minutes. Moreover, the proposed technique is rapid and reliable, thereby facilitating identification of *Enterobacteriaceae*. It is noteworthy that the Berthelot color reaction may be advantageous in demonstrating urease

activity of organisms which hydrolyze urea much more slowly than does *Proteus*. For example, ammonia was detected from single-colony isolates of *Aerobacter aerogenes* after as little as 15 min of incubation in aqueous urea prior to color development. Application of the Berthelot color reaction to members of the paracolon group is currently under investigation in our laboratories.

This investigation was supported in part by grants from the U.S. Public Health Service, Baxter Laboratories, and the Attending Staff Association of the Los Angeles County General Hospital (Unit 2).

SEROLOGICAL ASSAY OF CULTURE FILTRATES FOR *STAPHYLOCOCCUS* ENTEROTOXIN

SIDNEY J. SILVERMAN

U.S. Army Biological Laboratories, Fort Detrick, Frederick, Maryland

Received for publication 29 November 1962

The agar diffusion technique of Oudin (Compt. Rend. **222**:115, 1946) has been used by Surgalla et al. (*J. Immunol.* **69**:357, 1952) to determine the relative concentrations of antigens in preparations of staphylococcal enterotoxin and to study its antigenic properties (*J. Immunol.* **72**:398, 1954). Bergdoll et al. (*J. Immunol.* **83**:334, 1959; *Arch. Biochem. Biophys.* **85**:62, 1959), using the same technique, identified a precipitate band with the emesis-producing component of partially purified toxin and used this procedure as a guide in the purification of enterotoxin.

Since highly purified type B enterotoxin produced by the S6 strain of *Staphylococcus aureus* was available, estimations of enterotoxin by the gel diffusion and quantitative precipitin test were compared.

Purified enterotoxin prepared by E. J. Schantz, Fort Detrick, by a modification of the procedure of Bergdoll (*Arch. Biochem. Biophys.* **85**:62, 1959) was dissolved in the desired concentrations in 0.02 M phosphate-buffered saline (pH 7.4) containing Merthiolate (1:10,000). Antitoxin was obtained by the extensive immunization of rabbits with this preparation, emulsified in Freund's complete adjuvant. The initial dose of 37.6 μ g was increased by fivefold amounts until

a maximal dose of 3.0 mg was administered. Each rabbit received a total of 19.5 mg of toxin over a 6-month period. All injections were given by the

TABLE 1. Comparison of *Staphylococcus enterotoxin* determinations by gel diffusion and the quantitative precipitin test

Sample	Dilution	Enterotoxin (μ g per ml)	
		Gel diffusion	Precipitin
B		143.0	153.8
B	1:5	150.0	
A		57.5	90.8
A	1:5	48.0	
F		180.0	169.6
F	1:5	173.5	
C		19.0	24.8
1		24.5	27.6
2		48.0	46.8
3		37.0	33.6
5		44.0	41.6
7		31.5	35.6
OA		15.9	22.4

subcutaneous route; the time interval and dose were dependent upon the reaction of the individual rabbit. After each injection the animals responded with an increase in body temperature

and a loss of weight. The pooled sera contained 0.68 mg of antibody N per ml as determined by the quantitative precipitin test of Heidelberger and Kendall (J. Exptl. Med. 50:809, 1929).

Standard curves for both the gel diffusion test and the precipitin test were prepared with the purified toxin and antitoxin. Agar diffusion plates were incubated at 30 C in a glass-walled water bath. The movement of the precipitate band was measured at about 24-hr intervals, using a cathetometer. A single band of precipitate was formed in the interaction of these two reagents. For quantitative precipitin tests, the preparations were incubated at 37 C for 4 hr and then at 4 C for 4 days. Washed precipitates were analyzed for total N by the micro-Kjeldahl technique. Tests of the supernatant serum obtained after centrifugation and removal of the

precipitate never showed the simultaneous presence of both antigen and antibody.

The comparative tests were performed on a number of filtrates of *S. aureus* cultures grown in various media at 37 C for 18 to 24 hr. The cultures were centrifuged and the clarified fluid was sterilized by filtration through an ultrafine sintered-glass filter. Undiluted filtrate or filtrate diluted 1:5 with the buffered saline was tested by the Oudin test. In the quantitative precipitin tests, 0.5-ml amounts of undiluted or diluted filtrate (1:5) were mixed with 0.5 ml of antitoxin.

Representative results are presented in Table 1. Analysis of the data by the *t* test showed no significant difference between the results obtained with the two tests at the 95% level. These tests indicated that excellent correlation existed between the results obtained by the gel diffusion and the quantitative precipitin tests.

PROBLEMS IN THE USE OF EMULSIFYING AGENTS WITH WATER-INSOLUBLE SUBSTRATES¹

A. C. PETERSON² AND J. J. JEZESKI

Departments of Dairy Industries and Microbiology, University of Minnesota, St. Paul and Minneapolis, Minnesota

Received for publication 1 December 1962

When insoluble lipids are used as substrates in metabolic experiments, problems are met in providing excess substrate or sufficient substrate surface for enzyme action so that the activity measured is proportional to enzyme concentration. Emulsions of the lipid materials in water or buffer are frequently used in such experiments. To provide maximal dispersion of the water-insoluble phase and maximal stability of the emulsion, it is a common practice to add a stabilizer in the preparation of the emulsion.

In enzyme studies where stabilizers are added to substrate emulsions, there is always the question of whether the stabilizer has affected the rate of enzyme action and, if oxygen consumption is being measured, has contributed to oxygen uptake. In an investigation of unsaturated fatty

acid metabolism by bacteria, the emulsions of unsaturated fatty acids and their esters in distilled water or dilute buffer solutions, which were used as substrates in these studies, did not possess the desired stability for a sufficiently long period; therefore, the use of a stabilizing agent, Tween 80 (Atlas Powder Co., Wilmington, Del.), was considered.

A culture of *Pseudomonas fluorescens* (P-70) from the culture collection of the Dairy Bacteriology Laboratory of the University of Minnesota was grown on Tryptone (Difco)-glucose-meat extract agar for 24 hr at 20 C. The culture was recovered by washing off the surface of the Roux bottle flats with 10 ml of chilled 0.0031 M Sorensen's phosphate buffer (pH 7.4). The suspension of bacterial cells was centrifuged at 600 × *g* for 30 min in an International (size 2, model V) centrifuge. The supernatant fluid was decanted, and the cells were resuspended in more buffer and recentrifuged. The washing process was repeated four times, after which the cells

¹ Published with the approval of the Director of the Minnesota Agricultural Experiment Station.

² Present address: Department of Microbiological Research, Campbell Soup Co., Camden, N. J.