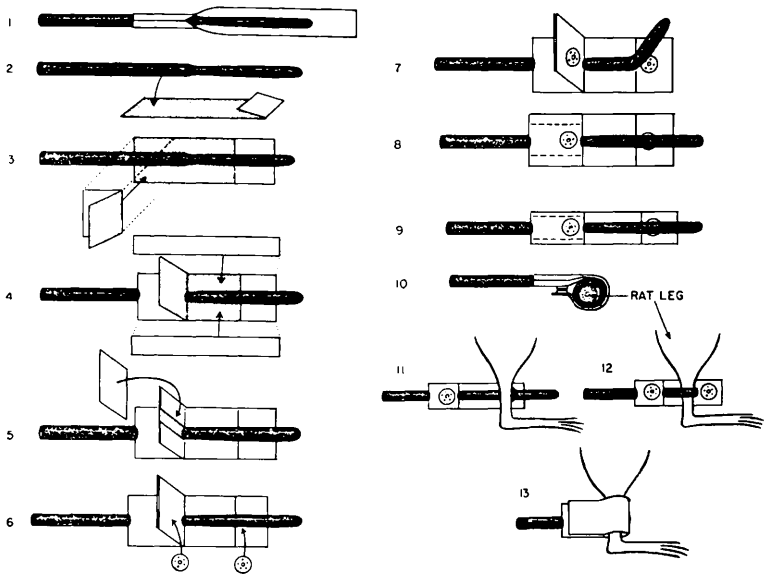

FIG. 1.

1. Commercial animal cuff for use with tensometer. Strip off the ribbon.
2. Place the bare rubber onto a piece of adhesive tape. Dots indicate sticky side of adhesive tape.
3. Cover part of non-inflatable end of the cuff with another strip of tape. For 150 g rats about 2.5 cm of inflatable cuff should be stuck to tape.
4. Cover exposed adhesive sections with 2 strips of tape.
5. Cover flap with a strip of adhesive tape.
6. Sew on a pair of small size snap fasteners. For 150 g rats, the snaps should be about 3.0 cm apart. 7-8. Fold down flap and sew. 9. Trim to size.
10. Top view of cuff in position.
11. Hold cuff so that free inflatable part is behind rat's leg.
12. Wrap free inflatable part of cuff around rat's ankle.
13. Snap the cuff shut, and rotate the entire cuff around rat's leg to insure even wrapping of inflatable portion.

within 3 mm.

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Factors Influencing the Plate Method for Determining Abundance of Bacteria in Sea Water.*† (23487)

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Total number of bacteria in sea water as determined by plate counts is influenced by numerous factors(1). In this report some effects of medium composition and plating procedure are described.

Materials and methods. Sea water was collected at Long Branch, N. J., approximately 600 feet off shore in an area subject to neither pollution nor dilution. All samples were taken at high tide, packed in ice, and transported

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TABLE I. Influence of Composition of Nutrient Medium on Plate Counts of Bacteria from Sea Water.

Medium constituents	Medium % composition* 1	2	3	4	5	6	7
Peptone	.5	.1	.5	.5	.5		.5
Gelatin						.3	
Dextrose		.1					
Glycerol					1.3		
Yeast extract			.01				
Beef extract				.3			
Ferric ammonium citrate				.01	.01	.01	
Na_2EDTA							.015
K_2HPO_4		.005		.01	.01	.01	.01
$FePO_4$	.01		.01				
$FeCl_3$							.002
Agar	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Plate count as % of medium 1 †	100	6.2	123.5	36.6	47.4	20.5	38.9

* In 80% sea water. † Mean of 8 replicates.

to the laboratory for study. The water had a salinity of 2.9-3.0% and a reaction of pH 7.9-8.1. Dilution blanks were prepared with aged sea water, sterilized by autoclaving. Appropriate dilutions of the water samples were plated, using 8 replicate plates at each dilution. Counts were made after 7-10 days of incubation at 20°C.

Results. Table I shows the extent to which composition of the nutrient medium influenced the plate counts. The results are expressed as percentages of the average plate count obtained with medium 1. Medium 1 was described by ZoBell(2) and is used frequently in studies of the abundance and distribution of bacteria in marine environments. Medium 2 was recommended by Reuszer(3) for the cultivation of marine bacteria. The remaining five media were our own formulations. Medium 3 is medium 1 supplemented with yeast extract. Media 4-7 contained different organic substrates, but were similar in that phosphate was added as K_2HPO_4 and iron was present as the citrate or ethylenediaminetetraacetate chelate. All the media were approximately pH 7.5.

Maximum plate counts were obtained on medium 3. Medium 2 yielded the lowest counts. The increased count that resulted from the addition of yeast extract to ZoBell's medium confirms Jones' report(4) that yeast extract is "stimulatory" to marine bacteria.

Although medium 3 gave maximum plate counts, it contained a precipitate ($FePO_4$), the particles of which were easily confused with the smaller subsurface colonies. The enumeration of colonies on medium 3 was facilitated greatly when the liquified agar was left undisturbed until the precipitate settled, and only the clear supernatant was used for the preparation of plates. In media with chelating agents (media 4-7) difficulties due to precipitation were absent but plate counts were low. When medium 3 was prepared using distilled water, the plate counts were only 10% of those obtained when the medium was prepared with sea water.

The plating procedure also influenced the total number of colonies that developed from sea water on medium 3. Pour plates were prepared by adding agar to Petri dishes containing 1.0 ml of various dilutions of sea water. The medium and sea water were mixed thoroughly before the agar solidified. A second series of Petri dishes was prepared by adding approximately 15 ml of medium to each plate and allowing the agar to solidify. The surface of the agar was then inoculated with 0.1 ml of various dilutions of sea water, and the inoculum was distributed over the surface of the agar by rotating and tilting each plate. Petri dish tops made of metal and containing ab-

sorbent disks were used to desiccate the agar surface partially and thus to prevent spreading of colonies.

The number of bacterial colonies that developed on pour plates was consistently greater (30-40%) than the number that developed on surface-inoculated plates. This difference may have resulted from the growth of microaerophilic or anaerobic bacteria below the surface of the agar in pour plates. Colony counts were made more easily when a surface inoculum was employed; the colonies were larger, more uniform in size, and better distributed than those on pour plates.

Summary. The nutritional requirements of marine bacteria are so varied that no one nutrient medium can suffice for the growth of all. The influence of composition of the nutrient medium and plating procedure on the plate method for determining the number of bacteria in sea water was investigated. Seven media were tested. Maximum and reproducible plate counts were obtained with a medium containing 0.5% peptone, 0.01% yeast extract, 0.01% $FePO_4$, and 1.5% agar in 80% sea water. The number of colonies that developed on pour plates was 30-40% greater than the number that developed on surface inoculated plates.

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Attempts to Produce Antibodies to a Preparation of Polyglutamic Acid.* (23488)

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One of the recently proposed plasma expanders produced by synthetic methods has been poly-L-glutamic acid. Although many data have been obtained on the physico-chemical and physiological properties of synthetic polyamino acids(1), studies on the antigenicity of the polyamino acids themselves have been rather scant. The recent investigations of Stahmann and coworkers on the antigenicity in rabbits and guinea pigs of various polypeptides (polymers, copolymers and multico-polymers) either uncoupled or coupled to proteins (polypeptidyl proteins) are extremely significant and interesting(2,3). This report presents data concerning unsuccessful attempts to produce detectable antibodies in man, rabbits and guinea pigs with a preparation of poly-L-glutamic acid different from the one employed by Stahmann, *et al.*

Materials. The sodium poly-L-glutamate (PGA) (Lot No. S2035-186C) used in these studies was prepared by the American Cyanamid Company according to procedures of Dr. E. R. Blout and coworkers. The weight average molecular weight of the material as determined by viscosimetric and light scattering technics was approximately 80,000 which is equivalent to a degree of polymerization of 620. The preparation of polyglutamyl bovine plasma albumin (#693) PGA-BSA which was also used in testing the various sera, as described below, was kindly supplied by Dr. M. Stahmann. Analytical solutions of the above materials were prepared by dissolving the materials in pH 7.4 buffered 0.15 M NaCl and analyzing aliquots for N by the Markham modification of the micro Kjeldahl procedure(4).

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