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.

The technical assistance of Miss E. Winkler is gratefully acknowledged.

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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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TABLE I. Schedule of Injections of PGA into Human Volunteers.*

Group	No. of volunteers	Amt of PGA inj. per inj. (mg)	No. of inj.
1	6	2	5
2	6	5	5
3	6	15	5
4	6	50	5

* Saline solutions inj. intramusc.
No significant skin reactions were observed.

Methods. 1. *Immunization procedures*: a. *Man.* Healthy medical student volunteers were used. The procedures employed for obtaining pre- and post-immunization bleedings, and performing intracutaneous skin testing were those previously presented(5). The volunteers received intramuscular injections of varying concentrations of the materials over a period of 8 days as summarized in Table I. Post injection bleedings were obtained 3 weeks after the last injection. All serum samples were handled with sterile precautions and in addition "merthiolate" (0.01%) and phenol (0.25%) were added. b. *Rabbits.* Various immunization technics with alum precipitated PGA, or the PGA incorporated in complete adjuvant vehicle(6) were employed. For immunization with alum precipitated PGA (1.5 mg/ml) the schedule of 4 intravenous injections per week for 4 weeks as described in (7) was employed. The adjuvant preparation contained 25 mg PGA per ml. Rabbits received intramuscular injections of 1 ml of the material 3 times per week for 3 weeks. Cardiac bleedings were performed weekly for 8 weeks before reinjections of the PGA were resumed. The schedule of reinjections is presented in Table II. c. *Guinea pigs*: 1. Five guinea pigs (300-400 g) were given 1 ml injections (1 mg PGA/ml) intraperitoneally every other day for a total of 3 injections. Another group of 4 guinea pigs was given one intracardiac injection of 5 mg PGA and 3 other guinea pigs received one intracardiac injection of 10 mg PGA. Twenty-two days after the last injection all the guinea pigs were challenged with 10 mg PGA. The route of injection was either by cardiac puncture or injection into the foot or penile veins. 2. Six guinea pigs were given two 0.5 ml injections of the adjuvant mixture mentioned above. Bleedings were taken weekly for 8 weeks starting 3 weeks after the last injection. 2. *Testing of Antisera*: a. Agar Diffusion—All of the pre and post injection sera from rabbits and humans were analyzed both by the Oudin and Ouchterlony procedures of diffusion in agar(8). In the Oudin procedure the gellified media consisting of equal volumes of undiluted serum and 0.6% agar were overlayered with PGA (0.5 mg N/ml), PGA-BSA (0.5 mg N/ml), BSA (0.7 mg N/ml) or 0.15 M NaCl. The tubes were placed in a constant temperature room (20°C) and observed for 2 weeks. In the Ouchterlony procedure undiluted serum was placed in the center well and two-fold dilutions of PGA (2 mg/ml) were placed in the 6 surrounding wells. With PGA-BSA the concentrations employed ranged from 2.5 μg N to 124.0 μg N/ml. The diffusion plates were placed in the cold room and observed daily for 2 weeks. In addition to the above sera, a serum kindly supplied by Dr. M. Stahmann was also included in the experiments. b. Quantitative Precipitin Studies. 3.0 ml aliquots of the sera were set up with varying concentrations of polyglutamic acid (1.37 μg N to 12.5 μg N) and PGA-BSA (2.6-42.4 μg N). The mixtures were incubated at 37°C for ½ hour and placed in the cold for 2 weeks. The tubes were then centrifuged and analyzed for antibody by the quantitative precipitin method of Heidelberger and MacPherson(9). c. Passive Transfer Studies. 1. 1 and 2 ml aliquots of several pools of human and rabbit sera were injected intraperitoneally into guinea pigs. Forty-eight hours later they were injected intravenously with 5 mg PGA. 2. The passive cutaneous anaphylaxis reac-

TABLE II. Schedule of Injections of Rabbits with PGA.

		Methods of immunization		
		Course		
Group	Rabbits	I	II	III
I	6	Alum*	Alum	Adjuvant
II	6	Adjuvant†	Adjuvant	

All rabbits bled weekly for 8 wk before reinjections began.
* 1.5 mg PGA/ml. † 25 mg PGA/ml.

tion (PCA) as described by Ovary(10) was also attempted employing 12 guinea pigs (200-250 g). 0.1 ml volumes of the undiluted antiserum and 1→5 and 1→10 dilutions were injected intradermally in different sites. Four to 6 hours after the intradermal injection, 1.0 ml of a dye solution (0.25 ml 1% Evans Blue and 0.75 ml saline) was injected intravenously. Thirty minutes later a solution of PGA (1 mg) and Evans Blue was injected intravenously.

Results. Although positive reactions in agar were observed with the serum obtained from Dr. Stahmann and PGA-BSA, detectable antibodies were not observed in any of the sera obtained in this study by the technics of diffusion in agar, micro precipitin and passive anaphylaxis. In addition none of the guinea pigs "immunized" with PGA exhibited active anaphylaxis or cutaneous reactions when challenged with PGA.

Discussion. In view of what we know about the antigenicity of other materials such as pneumococcal polysaccharides(11), dextran(12), levan(13) and polyvinyl pyrrolidone(14), one is not surprised at the results obtained by Stahmann on the antigenicity of his preparation of PGA. However, the results presented in this report were quite unexpected. Of the possible factors contributing to the "non-antigenicity" of Blout's preparation of PGA the most plausible one appears to be the difference in the properties of the 2 polyglutamic acid preparations. The method of synthesis employed by Blout(15) results in a linear preparation with a molecular weight of 80,000, whereas Stahmann's preparation is branched and has a molecular weight of 13,000. It is not known how these differences in properties of the two preparations account for the differences in results. Further investigations with other polypeptides having different physico-chemical properties are necessary to answer this question.

Summary. Data have been presented to show that detectable antibodies were not observed in man, rabbit or the guinea pig against a preparation of polyglutamic acid. The differences in properties between Blout's and Stahmann's PGA have been mentioned as the possible reason for differences in antigenicity of the two preparations.

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