source of dietary protein. Addition of 4 specific amino acids to raw soybean diet corrects poor growth and reduced food efficiency but does not prevent pancreatic hypertrophy. These results support the concept that decreased growth rate and protein efficiency caused by feeding raw soybean meal are due to direct stimulation of the pancreas resulting in excessive loss of critical amino acids contained in pancreatic enzymes excreted in feces.

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A Latex Agglutination Test for Anaerobic Diphtheroids. (25951)

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Serological observations with anaerobic diphtheroids have been limited because of the tendency of these organisms to grow in clumps and to agglutinate spontaneously. This communication is to report a latex (polystyrene) agglutination test for anaerobic diphtheroids which is reproducible, easy to read and not influenced by spontaneous bacterial agglutination.

Materials and methods. Organisms were characterized as anaerobic diphtheroids if they required an anaerobic environment for multiplication, had a pleomorphic rod-like or branching appearance, were Gram positive, produced catalase and did not ferment xylose, salicin or raffinose(1). All of our anaerobic diphtheroid strains were recovered and maintained in fluid thioglycollate medium. Most of the other bacteria mentioned in the text were obtained from the American Type Culture Collection and maintained in appropriate media. *The latex test* required an antigen-latex-buffer mixture which was prepared by mixing a suspension of washed bacteria (density to match McFarland #8 standard), 0.81 μ latex particles and glycine saline buffer (pH 8.2). (One part of the stock suspension of latex was diluted with 4 parts of distilled water to give the proper concentration of latex.) To prepare 5 ml of this mixture: 0.5 ml of bacterial suspension, 0.2 ml of diluted latex and 4.3 ml of buffer were mixed and allowed to stand at room temperature for at least 10 minutes. Sera which were to be tested were diluted 2-fold starting with a 1:10 dilution in glycine saline buffer to yield 0.5 ml in each tube. Clear test tubes measuring 10 x 75 mm were used. To each tube of diluted serum, 0.5 ml of the prepared antigen-latex-buffer mixture was added. Tubes were shaken, incubated for 1½ hours in 56°C water bath, centrifuged at 2300 rpm for 3 minutes and read. Appropriate nonspecific serum and antigen controls were included. Positive reactions were indicated by a clear supernatant, negative reactions by a turbid supernatant similar to that in the control tubes. *In bacterial agglutination test,* the same bacterial antigen was used

as in the latex test. Serial dilutions of sera were made. To 0.2 ml of each dilution, 0.2 ml of antigen was added. The mixtures were incubated at 37°C for 2 hours, then left overnight at 4°C. Agglutination was read after gentle shaking.

Results. 1) *Investigation of conditions required for a satisfactory latex test. pH* of the system was found not to be too critical. The test worked well within pH range of 5 to 10. Below pH 4, spontaneous agglutination of the latex particles occurred. *Temperature of Incubation,* whether at 37°C or 56°C, seemed to have little or no effect upon the results when tests were read after 1½ hours. *Size of latex* (polystyrene) particles was critical. Reactions that were clear-cut when particles with a diameter of 0.81 μ were used, failed to appear when particles of 0.1 μ diameter size were substituted. (These particles were furnished by Dr. Jacques Singer.) *Amount of latex* used in each test had to be adjusted. If there was too little or too much, tests were difficult to read. Satisfactory *antigens* were saline-washed bacteria from thioglycollate cultures incubated at 37°C for 3-7 days. Several of these anaerobic diphtheroids agglutinated spontaneously when used as an antigen in the bacterial agglutination test, but when used in the latex test this difficulty did not influence the results. Some antigen could also be found in the supernatant of a saline suspension of these bacteria kept at 37°C overnight. Both these antigens were destroyed by boiling. However, if the bacterial suspension was first acidified, an extract could be prepared by boiling, as in the Lancefield method for streptococci(2). This extract contained an antigen which also reacted in the latex test with serum from a rabbit immunized with whole organisms. *Amount of antigen* had to be controlled. For example, in one experiment with the standard amount of bacterial antigen, the titer of a serum was 1/320; whereas when 3 times that amount of antigen was employed the titer fell to 1/80, and with ⅓ the amount of antigen, the reaction was unreadable. *Order of addition of reagents* was very important. Latex particles had to be first sensitized with the antigen—not with the immune serum.

TABLE I. Serological Reactions of Anaerobic Diphtheroids with Homologous and Heterologous Rabbit Antisera.

	Diphtheroids			
	M		G	
Serum	Aggl.	LA	Aggl.	LA
Normal	0	0	0	0
Anti-M	1/160*	1/160	0	0
Anti-G	0	1/160	0	1/640

Aggl. = Bacterial agglutination test.
LA = Latex " " .

* Highest dilution of serum giving positive reaction.

2) *Demonstration of immunological relationships by means of the latex test.* An example is shown in Table I. Rabbits were immunized by intravenous inoculation twice weekly of increasing amounts of suspensions of anaerobic diphtheroid strains, G or M, which had been recovered from patients at the North Shore Hospital. (Strain G had been isolated from the bone marrow of a child with an osteolytic bone lesion, and strain M had been recovered from the blood stream of an adult with arthritis and pericarditis.) When these strains were tested by means of the bacterial agglutination test with specific antisera only the M strain was agglutinated by the anti-M serum. G was not agglutinated by its homologous antiserum. However, when these organisms were examined by latex agglutination test, both G and M strains gave positive reactions with their homologous antisera. The anti-G serum also cross reacted with the M organism, although at a lower titer. This suggested the possibility of an immunological relationship between both anaerobic diphtheroids. Indeed, with the latex agglutination test it was possible to demonstrate an immunological relationship between many anaerobic diphtheroids. Table II summarizes the results of several experiments with the latex test in which anti-G serum was used with a variety of anaerobic diphtheroids and related bacteria. All of the typical anaerobic diphtheroids tested gave positive reactions. However, some strains after continued passage lost their ability so to react. As for the positive reaction with *C. acne,* others, using different technics(1) have also found that this organism may cross react serologically with anaero-

TABLE II. Results of Latex Agglutination Tests between Anti-Anaerobic Diphtheroid Strain G Rabbit Antiserum and a Variety of Microorganisms.

Organisms	Results	Organisms	Results
Anaerobic diphtheroids		Actinomyces	
G	+	*A. bovis*	0
M	+	*israeli*	0
E	+		
R_O	+	Others	
T	+	*Nocardia asteroides*	0
R_B	+	*Lactobacillus acidophilus*	0
MOR	+	*Propioni bacterium freudenreichii*	0
Corynebacteriae		*Staphylococcus aureus*	0
C. acne	+	*Beta hemolytic streptococcus* (type 12)	0
hoagii	0	*H. influenza* (type B)	0
xerose	0		
diphtheriae	0		
pseudodiphtheriticum	0		
pyogenes	0		

bic diphtheroids with which it probably shares a common antigen.

Discussion. Latex (polystyrene) particles which are now readily available have become useful serological tools. Singer and Plotz(3) first employed them in a test for rheumatoid arthritis. More recently, there have been reports of their value in study of histoplasmosis (4) and of trichinosis(5). These particles probably make more apparent a precipitinogen-precipitin reaction. Collodion particles were used for this purpose more than 30 years ago, but were of limited value since conditions for preparing and preserving them were very exacting and time consuming(6).

Singer and Plotz(3) stated that the "latex fixation" technic could also be used with Type VII antipneumococcal rabbit serum and Type VII pneumococcal polysaccharide. In this report, we have extended this observation to another bacterial antigen-antibody system, that of anaerobic diphtheroids and their specific antisera, and have investigated optimum conditions for this reaction. Other bacterial antigen-antibody systems, however, may require different conditions.

Summary. A technic for using latex (polystyrene) particles in a serological test with anaerobic diphtheroids is described. Optimum conditions under which latex particles may be used with this bacterial antigen-antibody system are reported. Evidence is presented to indicate a possible immunological relationship between various anaerobic diphtheroids.

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Effect of Phosphorylated Hesperidin and Hyaluronidase on Rate of Erythrocyte Removal from Rat Peritoneal Cavity.* (25952)

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Phosphorylated hesperidin decreases net absorption rate of 0.9% sodium chloride solution from the peritoneal cavity of rats. Hyaluronidase abolishes this effect and by itself increases absorption rate in intact animals(1). The investigations reported below were con-

* Opinions expressed herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or naval service at large.

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