ing factors. The data here presented failed to establish a relationship between the presence of meningococci in the throat and the inhibitory activity of the throat washings. This might be explained by recent evidence (8) that numerous meningococci in the human pharynx are intracellular. However, the anti-meningococcal activity of some of the throat washings tested was of such a high degree that the role of this substance in the initial colonization and subsequent elimination of the meningococcus from the pharyngeal cavity should be considered.

It was interesting to note that of the 4 groups of men examined which are shown in Table I, the recruits in December 1964 were experiencing 4-5 times as much upper respiratory viral infections as the other groups. The anti-meningococcal activity of the throat washings of the recruits in December was the highest of any group tested. The possible relationship of this observation with the increasing evidence (R. O. Peckinpaugh, *personal communication*) that meningococcus carrier rates in military camps with a high incidence of upper respiratory infection are much lower than camps with a low incidence, requires further study.

Summary. A substance has been detected in the throat washings of military recruits that is bactericidal for Groups A, B, and C. *N. meningitidis*, related *Neisseriae*, and other Gram-negative and Gram-positive bacteria. The inhibitor appears to be different in its chemical properties from previously reported bacterial inhibitors present in unstimulated saliva. The inhibitor has been characterized as a low molecular weight (5000 or less) substance which is dialyzable, resistant to proteolytic and lipolytic enzymes, Biuret-positive, and Molish-negative. Activity of the inhibitor against the meningococci was destroyed by heating at 100°C for one hour.

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Quantitative Studies on γ_2 Anti-Dinitrophenyl Antibodies.* (31103)

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It was shown that guinea pig, slow moving, 7S γ_2 antibody can both sensitize heterologous skin for PCA and can fix complement but cannot sensitize homologous skin for PCA(1, 2,3). These experiments were designed to quantitate the complement fixing biologic activity of γ_2 anti-dinitrophenyl (aDNP) antibodies by determining the minimum amount of γ_2 antibody needed to lyse sensitized tanned sheep erythrocytes.

Materials and methods. Passive cutaneous anaphylaxis (PCA). PCA was carried out as previously described(2,4).

Passive lysis. Boyden's technique(5) was used to sensitize sheep erythrocytes. Stavitsky's method(6) applied to dinitrophenylated antigens as previously described(3) was followed. In brief, sheep erythrocytes were tanned and coated with DNP-bovine serum albumin, 37 groups/molecule (G/M) bovine

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serum albumin (BSA). This highly derivatized antigen gave more constant results than a poorly derivatized BSA(7). Antibody preparations were diluted in isotonic veronal buffer (8). Lyophilized guinea pig complement (Certified Blood Donor Service, Inc., Jamaica, N.Y.) twice adsorbed with normal sheep erythrocytes was diluted 1:15 in veronal buffer. Experimental tubes contained 0.5 ml of antibody dilution, 0.1 ml of 1% suspension of cells in veronal buffer, and 0.1 ml of diluted complement. Results were estimated by direct inspection, and recorded as follows: 4+, complete lysis, no turbidity; 3+, slight turbidity; 2+, obvious lysis but definite turbidity; 1+, small reduction in turbidity; 0, no lysis. The titer of a preparation was considered to be the greatest dilution which gave lysis of 2+ or more. Results were read after 45 minutes of incubation at 37°C.

Animals. Albino, Hartley strain guinea pigs of both sexes were used in all experiments. For immunization, pigs of 400 ± 50 g, for PCA reactions, pigs of 250 ± 50 g were used.

Antigens. Bovine gamma globulin (BGG, Armour, Kankakee, Ill., Lot X 30604), guinea pig albumin (GPA, prepared by starch block electrophoresis), and crystallized bovine serum albumin (BSA, Armour, Lot A 69805) were coupled with twice recrystallized dinitrophenyl sulphonic acid (DNPS, Eastman Kodak) according to the method previously described(9). Preparations of 55 G/M (DNP_{55}-BGG), 25 G/M (DNP_{25}GPA), and 37 G/M DNP_{37}BSA), respectively, were chosen from many conjugated antigens so prepared and were used for these experiments.

Immunization. Eighteen pigs were immunized with DNP_{55}BGG and another 18 pigs with DNP_{25}GPA. Each animal received 0.5 ml of emulsion divided among the 4 footpads. The immunizing material was made from equal volumes of Freund's complete adjuvant (Difco, Detroit, Mich.) and the antigen (2 mg/ml) diluted in saline (500 γ antigen protein/pig). On the 21st, 28th and 37th days following initial immunization, the animals were boosted with 0.5 ml of a 1 mg/ml solution of the antigen diluted in saline, divided into 4 intradermal injections on the pigs' backs. Seven days after the last boosting the animals were bled out under pentothal anesthesia (1 mg/kg).

Antisera. The antibody content of these sera was estimated both by PCA with DNP_{37}-BSA as challenging antigen and by passive lysis with DNP_{37}BSA coated tanned erythrocytes. All sera had an activity for both PCA and lysis at dilutions of 1:1000 or greater and were used as sources of γ_1 and γ_2 aDNPBGG or γ_1 and γ_2 aDNPGPA.

Separation of γ_1 from γ_2. Precipitation of globulins with ammonium sulfate followed by chromatography on diethylaminoethyl cellulose (DEAE)(10) were used according to the description in(11). To 50 ml of pooled sera were added 50 ml of saturated ammonium sulfate at room temperature to give a final ammonium sulfate concentration of 50%. The globulins so precipitated were spun down at room temperature at 10,000 RPM for 10 minutes and were then redissolved in 40 ml of saline. The precipitation was carried out 2 more times and the final precipitate was dissolved in 25 ml 0.005 M phosphate buffer, pH 8, and dialyzed overnight against the same buffer to remove any ammonium sulfate.

DEAE was equilibrated with several changes 0.005 M phosphate buffer, pH 8, over a one-week period. A DEAE column, 2 cm in diameter and about 40 cm in height, was then packed and flushed with the 0.005 M phosphate buffer until the pH and conductivity of the effluent and the affluent were equal. 500 mg of dialyzed gamma globulin was applied to the column and eluted at room temperature first with .005 M phosphate buffer. The protein concentration was measured at 280 lambda wave length in a Zeiss Spectrophotometer, model PM QII. An O.D. of 1.3 was taken to correspond to 1 mg protein/ml. Ten ml aliquots were collected until the $O.D._{280}$ readings were less than .20 (about 13 aliquots). Then the column was flushed with 400 ml of a .005 M phosphate buffer and similarly eluted at room temperature with 0.15 M phosphate buffer, pH 8. The respective aliquots with an O.D. reading greater than .20 were pooled and concentrated by pressure dialysis. From each sera, 2 samples were obtained: the first, eluted with 0.005 M buffer (which contained γ_2 antibody, see *Re-*

TABLE I. Optical Density of Globulin Eluates.*

Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Eluted with																		
.005 M†	.08	.04	.02	.04	.06	.01	2.7	1.6	.68	.39	.28	.20	.16	.15	.11	0	0	0
.15 M	0	0	0	0	0	0	1.9	3.5	3.0	2.1	1.3	1.3	1.1	.98	.61	.21	.16	.13

* Optical density measured at 280 lambda wave length. † Molarity of the buffer.

sults) and the second eluted with 0.15 M buffer (which contained γ_1 antibody, see *Results*). The concentrated material was then tested for PCA and lysis as described above. In all, 2 samples of γ_1 and γ_2 aDNPBGG and 2 samples of γ_1 and γ_2 aDNPGPA were prepared and tested for PCA and lytic activity. All γ_2 specimens were analyzed by the quantitative precipitin reaction of Heidelberger and Kendall(12). Finally, all γ_2 specimens as well as 2 preparations of isolated and purified γ_2 guinea pig aDNPBGG and aDNPBSA prepared as described in(13) were titrated by passive lysis as described above.

Results. Table I shows the $O.D._{280}$ of the eluted preparations of one of the experiments. Similar results were obtained in all the other experiments. The separation by chromatography on DEAE cellulose columns gave 2 preparations. Two distinct peaks were obtained, the first with the .005 M phosphate buffer and the second with the 0.15 M buffer. That these 2 different preparations contained, respectively, only γ_2 or γ_1 anti-DNP antibodies is shown by the PCA and passive lysis experiments on the concentrated material.

The results of a typical experiment to elicit PCA with γ_1 and γ_2 preparations are shown in Table II.

The results of a typical experiment to elicit passive lysis are shown in Table III. The fact that the γ_2 preparations (obtained by elution with the .005 M buffer) gave no PCA reaction at 1:10 dilution but lysed the $DNP_{37}BSA$ coated erythrocytes up to 1:30,000 dilution shows clearly that these preparations contained little, if any, γ_1 antibodies. On the other hand, the γ_1 preparations gave no lysis at dilutions of 1:10 but gave PCA reactions up to 1:20,000. Here again very little, if any, γ_2 aDNP antibodies were present in these preparations.

TABLE II. PCA with γ_1 and γ_2 Anti-DNP Antibodies.

Sensitizing antibody preparation*	Dilution	Reactions of 4 guinea pigs† 1	2	3	4
γ_1	1:100	25	25	23	27
γ_1	1:1000	20	18	20	22
γ_1	1:5000	18	16	14	18
γ_1	1:10,000	13	0	10	12
γ_2	1:10	Tr‡	0	0	0

* 0.1 ml injected into each skin site.
† Challenging antigen was 500γ per pig of DNP_{37} BSA. Results tabulated are the reaction diameters in mm.
‡ Tr = trace.

TABLE III. Passive Lysis with γ_1 and γ_2 Anti-DNP Antibodies.

Antibody preparation	Dilution	Lysis*
γ_1	1:10	0
γ_2	1:100	4+
γ_2	1:1000	4+
γ_2	1:2000	4+
γ_2	1:4000	4+
γ_2	1:8000	4+
γ_2	1:16000	4+
γ_2	1:32000	4+
γ_2	1:64000	0
γ_2	1:128000	0

* The meaning of the numbers is given in text.

A typical quantitative precipitin curve of a γ_2 specimen is shown in Fig. 1.

The results of the comparative titration of all 4 γ_2 preparations as well as the 2 isolated and purified antibodies diluted in veronal buffer or in normal guinea pig γ_2 (200 γ protein/ml) are shown in Table IV.

Discussion. It was previously observed that guinea pig aDNPBGG antibodies gave PCA reactions with α, epsilon-bis-DNP-lysine, whereas guinea pig aDNPBSA antibodies did not(14,15). As guinea pig aDNPBGG antibodies are generally of high affinity whereas aDNPBSA or aDNPGPA antibodies are of low affinity, it is interesting to note that only the high affinity antibodies are capable of giving the PCA reactions with this bivalent hapten. It was observed with rabbit antibodies

TABLE IV. Passive Lysis of γ_2 Anti-DNP Antibodies.
Different antibody preparations

Antibody concentration in μg of protein/ml	γ_2 aDNPBGG		γ_2 aDNPGPA		Isolated & purified γ_2 aDNPBGG		Isolated & purified γ_2 aDNPBSA	
	No. 1	No. 2	No. 1	No. 2				
Diluent	Veronal buffer		Veronal buffer		Veronal buffer	Veronal buffer + 200 γ/ml of normal GP γ_2 protein	Veronal buffer	Veronal buffer + 200 γ/ml of normal GP γ_2 protein
10	4+*	4+	4+	4+	4+	4+	4+	4+
5	4+	4+	4+	4+	0	3+	0	3+
2.5	4+	4+	4+	4+	0	0	0	0
1.25	4+	4+	4+	4+	0	0	0	0
.63	3+	4+	4+	3+	0	0	0	0
.32	0	4+	0	0	0	0	0	0
.16	0	0	0	0	0	0	0	0

* The meaning of numbers is given in text.

that low affinity antibodies needed more antigen to provoke PCA reactions than high affinity antibody(16,17). Furthermore, with highly derivatized DNP-protein as antigen in threshold quantities, the same difference already noted with rabbit antibody was also observed with guinea pig γ_1 antibodies, namely, more antigen was needed to provoke PCA reactions with low affinity antibodies. Moreover, when only a few groups of DNP were on the carrier protein (DNP_3BGG), even with excess of antigen, 4 times more low affinity antibody was needed to provoke threshold PCA reactions; however, with highly derivatized antigens in excess, no difference could be observed in PCA reactions between high and low affinity guinea pig γ_1 antibodies(18).

In the present experiments, highly derivatized DNPBSA (37 G/M) was used to coat tanned sheep erythrocytes. In preliminary experiments it was seen, in fact, that only highly derivatized antigen in the concentration indicated above could be used for adequate coating. When poorly derivatized DNPBSA or when lower concentrations of highly derivatized DNPBSA were used, the results were not reproducible and often complete lysis could not be obtained(7). If one could extrapolate from what is known of the biological behavior of γ_1 antibodies to γ_2 antibodies, one would not expect to be able to distinguish between high and low affinity antibodies with passive lysis using the above described experimental conditions. The nature

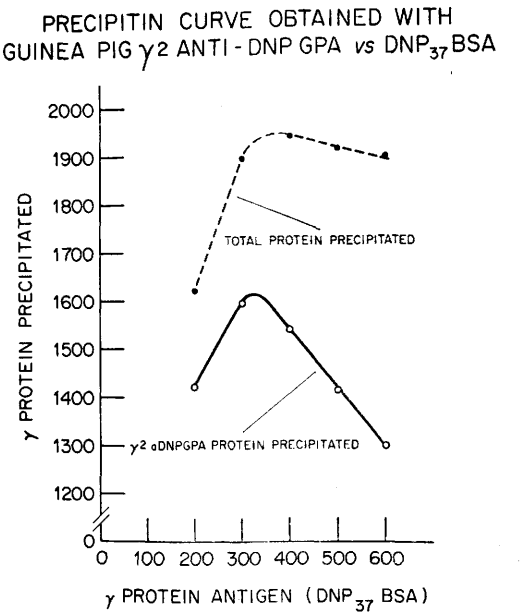

FIG. 1. To 1 ml of $\gamma 2$ preparation different of antigen dissolved in saline were added. Total vol. was 2.5 ml per tube.

of this type of experiment, *i.e.*, the use of excess of highly derivatized DNPBSA, creates conditions which do not permit distinction between high and low affinity antibodies. With excess of highly derivatized antigen no difference could be demonstrated between γ_1 antibodies of high and low affinities(18), and no difference was seen in passive lysis between guinea pig γ_2 high (aDNPBGG) and low (aDNPGPA) affinity antibodies. Both gave lysis with 0.3 to 0.6 γ antibody protein/ml.

This is of the same order of magnitude as the amount of antibody needed to provoke the PCA reaction(19).

Isolated and purified guinea pig aDNPBGG and aDNPBSA antibodies prepared as previously described(1) were also used for passive lysis. Although these specimens were stored for several weeks in the refrigerator, they still retained their full capacity to precipitate DNPBSA and had the same affinity as that found immediately after isolation. Even though aDNPBGG antibody had an affinity 10 times that of the aDNPBSA antibody, no difference could be detected using passive lysis. However, about ten times more of this isolated and purified antibody was needed (5γ antibody protein/ml) to provoke lysis than of the recently eluted and not isolated and purified γ_2 preparations. To minimize the effect of protein absorption on glass surfaces of highly diluted antibody preparations, these experiments were performed also in veronal buffer containing 200 γ of normal guinea pig γ_2 globulin/ml. However, no significant differences were found between the specimens diluted in veronal buffer with or without normal guinea pig γ_2 globulin. It was already noted with isolated and purified γ_1 antibody that storage in the refrigerator for any length of time diminished the biological activity(19). The same may also be true for isolated and purified γ_2 antibodies. In view of the fact that these antibodies quantitatively precipitate the same amount of antigen as when they were freshly isolated, it is probable that if any alteration occurred, it occurred on the Fc fragment, because the Fc fragment plays an important role in complement fixation(20,21,22), as well as in sensitization for PCA reactions (Skin "Fixation") (23).

Summary. Guinea pig γ_2 anti-dinitrophenyl antibodies can lyse tanned sheep erythrocytes coated with highly derivatized dinitrophenyl bovine serum albumin. With the method used, 0.3 to 0.6 γ antibody protein/ml is sufficient to provoke lysis. Since no difference was found between high and low affinity antibodies, this method can be used for the quantitation of guinea pig γ_2 anti-dinitrophenyl antibodies of high and/or low affinities.

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