(6) or surgical resection of this part of the stomach(7) reduced the response to maximal stimulation with histamine, suggest that potentiation between endogenous gastrin and exogenous histamine may occur.

The finding that pairs of stimulants of which histamine is one member, such as histamine plus cholinergic stimulants and histamine plus gastrin, produce potentiation cannot readily be reconciled with the hypothesis that histamine is the final common mediator for all forms of stimulation of acid secretion (5). If all agents act through release of histamine, the simplest assumption would be that they should act additively with histamine in the degree of stimulation achieved.

Summary. Histamine and gastrin extracts were given separately and in various combinations of doses to dogs with Heidenhain pouches. Most of the combinations of histamine plus gastrin gave higher rates of secretion of acid than could be accounted for on the basis of summation of stimulatory effects. The maximal rate of secretion of acid was higher with the combination of agents than with either agent alone. It is concluded that there is potentiation between exogenous gastrin and exogenous histamine in stimulation of gastric secretion of acid.

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Properties of Guinea Pig 7S Antibodies IV. Antibody Response to *E. coli* Bacteria.* (28583)

K. J. BLOCH, F. M. KOURILSKY, Z. OVARY AND B. BENACERRAF
Department of Pathology, New York University School of Medicine, New York City

Previous reports(1-6) characterized 2 types of guinea pig 7S antibody differing in electrophoretic mobilities and biological properties. Guinea pig $7S\gamma_1$ antibodies sensitized guinea pigs for passive systemic and cutaneous anaphylaxis, $7S\gamma_2$ antibodies did not. Guinea pig $7S\gamma_2$, but not γ_1, antibodies fixed complement *in vitro* in the presence of antigen and sensitized antigen-coated cells for lysis in the presence of complement.

The influence of certain methods of immunization on production of these 2 types of antibody has been defined(1,3). Guinea pigs hyperimmunized with hapten conjugates, protein or tissue cells emulsified in complete Freund's adjuvant invariably produced both $7S\gamma_1$ and $7S\gamma_2$ antibodies. However, guinea pigs hyperimmunized with hapten conjugates (dinitrophenyl-bovine gamma globulin) administered intraperitoneally without adjuvants produced primarily γ_1 antibodies. The present report is concerned with the finding that guinea pigs hyperimmunized with a particulate antigen, *Escherichia coli* bacteria, emulsified in complete Freund's adjuvant produced predominantly $7S\gamma_2$ antibodies.

Methods. Immunization. E. coli 0 111:B4 bacteria were grown as described(7). Bacteria were killed by heating at 60°C for 30 minutes, washed twice in saline and suspended at a concentration of approximately 5×10^{11} bacteria per ml. Equal volumes of complete Freund's adjuvant and bacterial suspension were emulsified and 0.1 ml injected into each footpad of twenty-five 350-400 g Hartley-strain guinea pigs. Thereafter, animals were injected intradermally at 4

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sites with 0.1 ml of a suspension containing approximately 5×10^{11} bacteria per ml, at intervals of 7 days. Animals were exsanguinated 7 days after the last of 5 booster immunizations. Arthus reactions were observed at the skin site of the 4th and 5th booster injection.

A second group of 5 guinea pigs was injected intravenously with 1 ml of stock suspension, followed at 3-day intervals by 3 additional intravenous injections of 1 ml of 5×10^9 bacteria.

A third group of 3 animals was injected intraperitoneally with the same dose of bacteria and at the same intervals as Group 2. Further immunization of Groups 2 and 3 was performed as above.

Serologic tests. Passive hemagglutination and hemolysis. To 5 ml of a 2.5% suspension of washed sheep erythrocytes in veronal buffer(8), were added 250 μg of *E. coli* 0 111:B4 polysaccharide; the reactants were mixed and incubated at 37°C for 2 hours. Cells were then washed once in veronal buffer and resuspended at a concentration of 0.5%. One-tenth ml of cell suspension was added to 0.5 ml aliquot of serial 2-fold dilutions (in veronal buffer) of inactivated and absorbed (2 ×, with sheep erythrocytes) antibody solutions, mixed and held at 5°C for 18 hours. Settling patterns were scored according to the criteria of Stavitsky(9). Cells were then resuspended, mixed with 0.1 ml of guinea pig complement solution (Certified Blood Donor Service, Jamaica, N.Y., lyophilized guinea pig serum, 1 ml reconstituted C′ diluted with 14 ml veronal buffer), and incubated at 37°C for 15 minutes. Hemolysis was estimated visually; the last dilution yielding close to 100% hemolysis was considered end point of the solution tested.

Complement fixation was performed as described(8). To 0.5 ml of antibody solution was added 0.5 ml of C′ solution (1 ml guinea pig serum diluted with 99 ml veronal buffer) followed by 0.5 ml of antigen solution containing 5 μg *E. coli* polysaccharide. Reactants were mixed, held at 0°C for 18 hours followed by the addition of sensitized sheep erythrocytes and incubation at 37°C for 30 to 60 minutes.

Passive cutaneous anaphylaxis (PCA) was performed in guinea pigs as described(10). Antigens used consisted of 500 μg of *E. coli* 0 111:B4 polysaccharide or 0.2 ml of a bacterial suspension containing approximately 1×10^{11} cells. A dose of 500 μg polysaccharide produced optimum PCA results with the only guinea pig serum containing skin-sensitizing antibodies (see below). Rabbit anti-*E. coli* antiserum served as positive control in most experiments.

The opsonizing effect of guinea pig antibodies on blood clearance of P^{32}-labeled *E. coli* by the reticuloendothelial system of mice was tested using the method of Benacerraf *et al.*(7).

Electrophoretic separation. Isolation of antibodies. Starch block electrophoresis was performed by the method of Kunkel(11). Eluted fractions were dialyzed against water containing 0.005 M phosphate buffer pH 7.5, lyophilized and subsequently resuspended in 1.0 ml saline. Serologic tests were performed on 0.1 ml aliquot of this solution; an initial 10-fold dilution was used for screening. The protein content of eluates was estimated by the Folin technique. Immunoelectrophoresis was performed as described(1) except that duration of electrophoresis was extended to 3 hours.

Guinea pig anti-*E. coli* polysaccharide antibodies were isolated by precipitation with polysaccharide and subsequent dissociation of the specific precipitate in 15% NaCl(8).

Density gradient ultracentrifugation was performed as described(12) using a salt gradient ranging from 21% to 7% NaCl solution. A guinea pig anti-DNP-BGG antiserum was used as a 7S marker(1,3).

Results. Fig. 1 compares the protein content of eluates obtained by starch block electrophoretic separation of 2 representative guinea pig anti-*E. coli* antisera as well as a guinea pig anti-dinitrophenyl-bovine gamma globulin, and a guinea pig anti-sheep erythrocyte antiserum obtained by identical methods of immunization with adjuvants. In the latter 2 sera, protein content is distributed about 2 peaks in the "slow" (γ_2) and "fast" (γ_1) region of the gamma globulin zone. The 2 *E coli* antisera demonstrate only a single

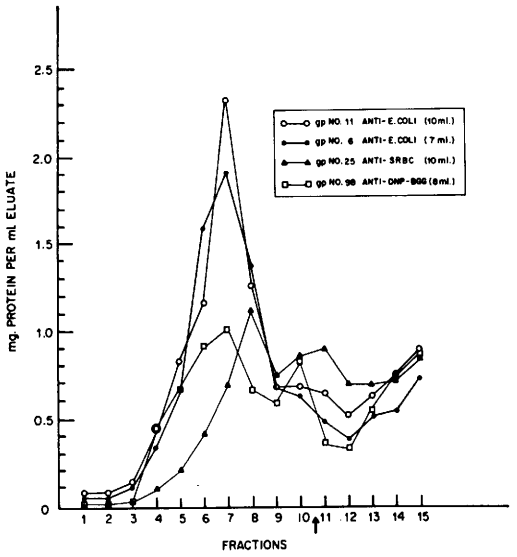

FIG. 1. Comparison of protein concentration of eluates from separate starch block electrophoretic separations of 2 guinea pig anti-*E. coli* antisera and a guinea pig anti-sheep erythrocyte and anti-DNP-BGG antiserum. (Anode, right; arrow indicates point of application of antisera on starch block.)

large peak in the "slow" or γ_2 region, but no well-defined peak in the γ_1 region.

Fig. 2 summarizes the results of tests performed on starch block eluates from one (serum #6) of several electrophoretically separated individual guinea pig anti-*E. coli* antisera. Antibody activity estimated with whole bacteria [determined by opsonic activity of fractions (A)] or its specific polysaccharide [determined by passive hemagglutination and hemolysis of antigen-coated erythrocytes (B), as well as by complement fixation (C)] is concentrated in the γ_2 region of the starch block. There is no indication of a second peak of hemagglutinating activity which might be attributed to γ_1 antibodies.

Density gradient ultracentrifugation was performed using aliquots of fractions 5, 6, and 7 of guinea pig anti-*E. coli* serum #4 (this serum had a starch block electrophoretic pattern comparable to that of serum #6, Fig. 2) mixed with a known 7S antibody marker(3). This experiment demonstrated that antibodies responsible for agglutination and lysis of polysaccharide-coated erythrocytes sedimented in the same zone of the gradient as the 7S marker; there was no additional peak of antibody activity in the faster sedimenting region of the gradient.

None of the fractions of guinea pig #6 anti-*E. coli* antiserum yielded a positive PCA reaction in guinea pigs challenged with *E. coli* polysaccharide or bacterial suspension. A total of 25 guinea pig antisera were tested for the presence of guinea pig skin-sensitizing antibodies. These sera had been demonstrated to precipitate with antigen (upon dilution of 50 μg polysaccharide to 0.5 ml serum), and to agglutinate and lyse antigen-coated erythrocytes in a final serum dilution of 1:1250

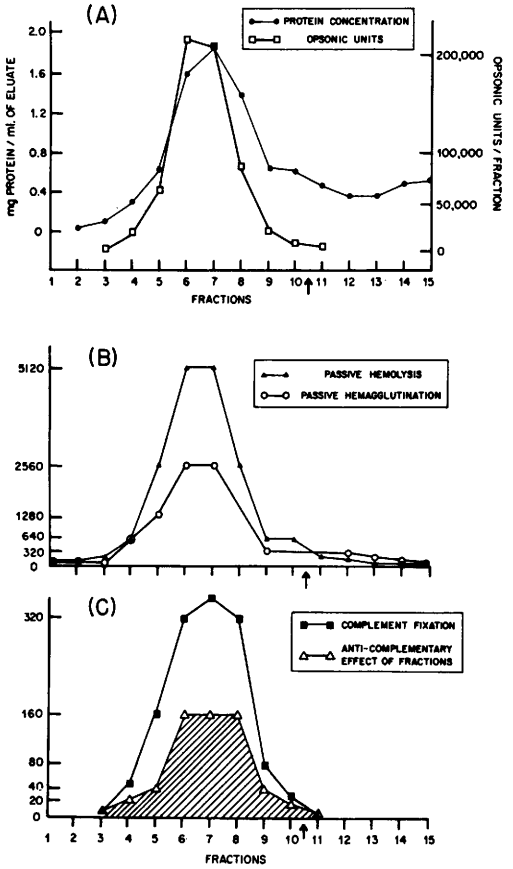

FIG. 2. Titration of protein concentration and opsonic activity (A), passive hemolysis and hemagglutination (B), and complement fixation and anti-complementary activity (C) of eluted fractions from starch block electrophoresis of guinea pig anti-*E. coli* antiserum #6. Note the marked anti-complementary effect presumably due to the high concentration of γ_2 globulins in these fractions. (Anode, right; arrow indicates point of application of antiserum on starch block.)

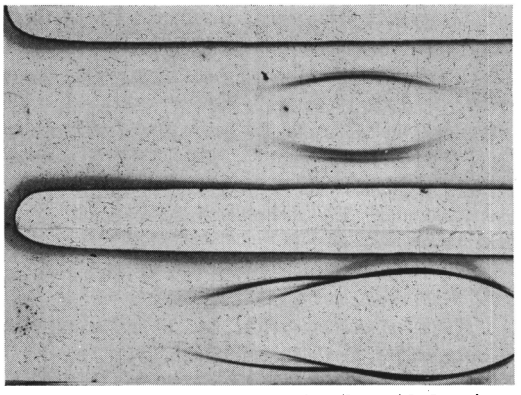

FIG. 3. Immunoelectrophoresis of purified guinea pig anti-*E. coli* polysaccharide antibodies (top well) and purified guinea pig anti-DNP-BGG antibodies (bottom well) developed with rabbit anti-guinea pig whole serum, R2, in troughs. (Anode, left.)

to 1:2500. However, 24 of the 25 sera failed to provoke cutaneous anaphylactic reactions in animals challenged intravenously with either *E. coli* polysaccharide or bacterial suspension; only one serum yielded a positive reaction up to a dilution of 1:100. Similarly prepared guinea pig anti-hapten or anti-protein antisera generally yield positive PCA reactions at a dilution of 1:2000 to 1:5000.

Fig. 3 compares the immunoelectrophoretic pattern of isolated guinea pig anti-*E. coli* polysaccharide antibodies with that of purified guinea pig anti-DNP-BGG antibodies. The former yields definite precipitin lines in the "slow" or γ_2 region with rabbit antiserum R2 [obtained from a rabbit immunized with pooled guinea pig sera(2)]; the latter yields 2 distinct precipitin arcs in the slow and fast region.

Discussion. Previous experiments have shown that guinea pigs hyperimmunized with hapten conjugates, protein, or tissue cells emulsified in adjuvant produced 2 types of 7S antibodies. Although $7S\gamma_2$ antibodies usually appeared earlier in the course of immunization, $7S\gamma_1$ antibodies were invariably produced by this method. A second type of immune response was found in animals hyperimmunized with hapten conjugates (DNP-BGG) administered without adjuvant. These animals produced primarily $7S\gamma_1$ antibodies. The present experiments demonstrate a third type of immune response in guinea pigs hyperimmunized with *E. coli* bacteria emulsified in adjuvants. These animals produced large amounts of $7S\gamma_2$ globulins but, with one exception, did not produce $7S\gamma_1$ anti-*E. coli* antibodies. Sera from hyperimmunized guinea pigs yielded only small amounts of precipitate with *E. coli* polysaccharide and, in moderately high dilutions, agglutinated and lysed antigen-coated erythrocytes. It is therefore difficult to account for the large amount of γ_2 globulins contained in these sera by antibody precipitable by *E. coli* polysaccharide. Although antibodies to other bacterial constituents might account for the remainder of the γ_2 antibodies, the possibility that immunization with *E. coli* emulsified in complete adjuvant non-specifically stimulates production of γ_2 globulins cannot be excluded.

The present study suggests that a property of the antigen is decisive in determining the type and amount of antibody produced. Of the various constituents of *E. coli* bacteria, the 0 somatic antigen or endotoxin appears most likely to be involved in, or responsible for, the γ_2 hyperglobulinemia observed, and possibly also for the failure to produce $7S\gamma_1$ anti-*E. coli* antibodies. Endotoxin has been shown to enhance antibody response in several species(13,14) but the guinea pig has been reported to be refractory to this enhancing effect when endotoxin is administered once or twice with antigen in doses ranging from 25 to 200 μg(13). In the present experiments, endotoxin as part of the antigenic mixture was, in effect, administered initially in Freund's adjuvant into the footpads and was subsequently injected intradermally with each booster 5 times at weekly intervals. Under these circumstances endotoxin may have potentiated the production of γ_2 antibodies or γ_2 globulins. Furthermore, repeated administration of endotoxin may, by continually recruiting cells involved in production of γ_2 proteins, interfere with the usual sequence of changes leading to the production of γ_1 proteins.

Previous studies have demonstrated the important role of specific antibody(7) and complement(14) in the blood clearance of *E. coli* by the reticuloendothelial system of

mice. The present study indicates that guinea pig γ_2 antibodies are effective opsonins in promoting immune clearance of *E. coli* bacteria. Finally, it should be noted that reliance on a single indicator of antibody formation, in this instance the PCA reaction, would have led to failure to detect the immune response in guinea pigs immunized with *E. coli* bacteria.

Summary. Guinea pigs hyperimmunized with *E. coli* bacteria emulsified in complete Freund's adjuvant produced large amounts of $7S\gamma_2$ globulins. However, animals immunized by this method, as well as others initially immunized with bacteria administered intraperitoneally or intravenously, failed, with one exception, to produce $7S\gamma_1$ guinea pig skin sensitizing anti-*E. coli* antibodies.

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Intracellular Site of Newcastle Disease Virus Nucleic Acid Synthesis.* (28584)

E. FREDERICK WHEELOCK (Introduced by J. H. Dingle)

Departments of Preventive Medicine and Medicine, School of Medicine, Western Reserve University, and University Hospitals, Cleveland, Ohio

Synthesis of Newcastle disease virus (NDV) protein has been demonstrated by immunofluorescence to take place in the cytoplasm of infected cells(1,2). Determination of the intracellular site of NDV nucleic acid synthesis, however, has been hindered by both failure to isolate infective NDV-RNA from cells and inability to differentiate synthesis of viral RNA from cellular RNA. An approach to the localization of NDV-RNA synthesis was suggested recently by the demonstration that several RNA-containing viruses, including NDV, can multiply to high titer in cells in which cellular DNA-dependent RNA synthesis has been inhibited by actinomycin D(3,4,5), and that NDV-RNA can be isotopically labeled in such cells(6).

In the experiments reported herein, actinomycin D† was used to differentiate synthesis

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† Actinomycin D was supplied by Dr. Karl Folkers, Merck Sharp and Dohme Research Laboratories.