tion indicate that a heterogenic hemagglutinin exists in pure parotid and submandibular fluids. This is supported by the findings of Beutner *et al*(19) who observed hemagglutination reactions in extracts of rabbit salivary glands. This substance may have been responsible for the apparent specific hemagglutinins (antibodies) described in previous investigations where adequate adsorptive procedures had not been utilized.

Summary. Human parotid fluid, submandibular fluid, and serum were assayed for tetanus and diphtheria antibodies. Significant increases in serum diphtheria and tetanus antibody titers occurred subsequent to toxoid administration. The antibody titers of pure parotid and submandibular saliva, however, remained constant. Saliva appears to contain a heterogenic substance which is able to agglutinate cells from horse, rabbit, dog, sheep, and man regardless of blood type or Rh factor.

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Inhibition by Lipopolysaccharide of Immune Phagocytosis of Latex Particles Modified with Common Antigen of Enteric Bacteria.* (30717)

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Numerous enteric bacteria, including *Escherichia, Aerobacter, Salmonella, Shigella,* and *Proteus,* share a common antigen (CA) (1,2). In view of the role of these microorganisms in infections of both man and animals, studies of the biologic and medical significance of this antigen-antibody system are of interest, for only limited information is available to date. It was shown recently that CA antibodies opsonize enteric bacteria for phagocytosis by polymorphonuclear leukocytes(3). Investigation of the bactericidal ac-

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tion of CA antibodies revealed that, in the presence of complement, killing of *E. coli* O14 was effected, but not that of other enteric bacteria sharing this antigen(3,4). The present study has demonstrated that CA antibodies opsonize antigenically modified latex particles for immune phagocytosis and, surprisingly, that lipopolysaccharide (O antigen) interferes with phagocytosis.

Materials and methods. The antigens used were derived from *S. typhimurium* and *Escherichia coli* O14 and O111. The microorganisms were grown in Kolle flasks on brain veal agar for 18 hours at 37° C. The growth was suspended in 25 ml of phosphate hemagglutination buffer (Difco; pH 7.3) per Kolle flask. The supernate obtained after centrifugation at 23,500 *g* was used as crude antigen and contained both CA and O antigen. CA was isolated and separated from the O antigen by means of 85% ethanol, as described previously (ethanol soluble fraction)(5). Highly purified *S. isangi* lipopolysaccharide was kindly made available by Professor O. Westphal and Dr. O. Lüderitz, Max-Planck Institut für Immunbiologie, Freiburg-Zahringen, Germany.

Antisera against CA were prepared in rabbits by repeated intravenous injections of *E. coli* O14 or of the ethanol soluble fraction from *S. typhimurium* or *E. coli* O111, according to previously described procedures(5). *Salmonella* O antiserum was purchased from Lederle Laboratories.

Polystyrene latex particles (Bacto-latex 0.81 μ, Difco Laboratories) were thoroughly washed 3 times in sterile, pyrogen-free saline. To the sediment from 1 ml, 2 ml of either CA in a dilution of 1:2, crude supernate, or mixture of CA and lipopolysaccharide was added. The mixtures were incubated in a waterbath at 37°C for 30 minutes. The antigenically modified latex particles were again washed 3 times in sterile saline solution and resuspended in Hanks' bovine albumin glucose solution.

Polymorphonuclear leukocytes were obtained from rabbit peritoneal exudates, according to the method of Cohn and Morse (6). Latex particles and leukocytes were mixed in a multiplicity of 4:1. Each phagocytic tube contained 1.6 ml leukocytes (30×10^6 per ml), 0.2 cc latex particles (960×10^6 per ml), and 0.2 ml of normal unheated rabbit serum or 0.2 ml of antiserum appropriately diluted in normal serum. Approximately 99% of the leukocytes were viable, as determined by the trypan blue dye exclusion test for leukocytes. The siliconized tubes were stoppered with sterile rubber stoppers and rotated, end-over-end, at 37°C at 8 rpm on a rollordrum-type rotator (New Brunswick Scientific Co., New Brunswick, N. J.).

Films, prepared at various times, were stained with methylene blue; 500 leukocytes were counted, and the number of leukocytes containing ingested latex particles was noted.

To prove that CA had become attached to latex particles, absorption studies were performed by mixing the sediment of treated latex particles and CA antiserum, incubating the mixture for 30 minutes at 37°C, and titrating the supernate with antigenically modified erythrocytes as indicator, according to the procedure previously described(7). CA used as indicator was obtained from enteric bacteria unrelated in O antigen to that used for immunization.

Results. Repeated experiments have revealed that latex particles modified with common antigen (CA) from *S. typhimurium* (ethanol soluble fraction) are opsonized by CA antiserum, as evidenced by increased phagocytosis when comparison is made with normal rabbit serum. The results of a typical experiment are shown in Table I. Surprising was the finding, also shown in the Table, that latex particles modified with the crude supernate of *S. typhimurium,* containing identical amounts of CA as the ethanol soluble fraction but, in addition, O antigen, were markedly less opsonized in the presence of the same CA antiserum. Experiments were undertaken, therefore, to determine whether the O antigen (lipopolysaccharide) interferes with phagocytosis by CA antiserum. The results of a representative experiment are shown in the lower portion of Table I. It can be seen that latex particles treated with a mixture of ethanol soluble CA and *S. isangi* lipopolysaccharide were not phagocytized in the presence of CA antiserum, but immune

TABLE I. Phagocytosis by Polymorphonuclear Leukocytes of Latex Particles Modified with CA, CA-Lipopolysaccharide Mixtures, or CA-Lipoid A Mixtures.

Antigens	Antisera	% leukocytes containing ingested latex particles Time (min) 0	30	60
CA (*S. typhimurium*)	CA (*E. coli* O14)	1	61	63
Idem	Normal rabbit serum	3	13	11
Crude (*S. typhimurium*)	CA (*E. coli* O14)	4	20	39
Idem	Normal rabbit serum	4	9	18
CA (*S. typhimurium*) and lipopolysaccharide (*S. isangi*, 200 μg)	CA (*E. coli* O14)	1	0	0
Idem	O (*Salmonella* C_1)	4	32	54
Idem	Normal rabbit serum	3	15	10
Lipopolysaccharide (*S. isangi*, 200 μg)	O (*Salmonella* C_1)	10	57	61
CA (*S. typhimurium*) and lipoid A (200 μg)	CA (*E. coli* O14)	4	30	31
Idem	Normal rabbit serum	5	16	12

TABLE II. Effect of Varying Amounts of Lipopolysaccharide on Immune Phagocytosis of Latex Particles Modified with Common Antigen.

Antigens CA (*S. typhimurium*) (1/2)	*S. isangi* lipopolysaccharide (μg)	Antisera	% leukocytes containing ingested latex particles Time (min) 0	30	60
CA	200	CA (*E. coli* O14)	6	2	3
CA	50	*Idem*	15	21	23
CA	12	*Idem*	28	39	50
CA	—	*Idem*	15	59	68
—	200	O (*Salmonella* C_1)	25	51	73

phagocytosis was observed with the corresponding O antiserum. Essentially identical results were obtained when latex particles were treated consecutively rather than simultaneously with CA and lipopolysaccharide, in either order. The results of the titration of the lipopolysaccharide as inhibitor of phagocytosis are shown in Table II and indicate that strong inhibition occurred with 200 μg, moderate with 50 μg, and minimal with 12 μg. It can be seen, too, that O antibodies effectively promoted phagocytosis.

Previous studies have established certain differences between CA from *E. coli* O14 and that from other enteric bacteria, although serologically the antigens are indistinguishable. Firstly, suspensions of *E. coli* O14 readily engender CA antibodies upon intravenous injection of rabbits in contrast to those of the others(1). Secondly, immunogenic CA from *E. coli* O14 is ethanol insoluble and that of the others ethanol soluble(8,9). Thirdly, *Pseudomonas aeruginosa* and psychrophilic *Pseudomonas* produce a factor which destroys selectively the ethanol soluble but not the ethanol insoluble CA(8,10,11). Phagocytosis experiments were, therefore, undertaken utilizing the ethanol insoluble fraction as well as crude supernate of *E. coli* O14, and it was observed that both preparations modify latex particles for opsonization by CA antibodies. Thus, another difference was established between CA from *E. coli* O14 and that of other enteric bacteria.

Experiments were then carried out to determine the effects of the lipoid A component of lipopolysaccharide on phagocytosis by CA antibodies. As shown in the lowest portion

of Table I, the simultaneous presence of lipoid A on the surface of the latex particles resulted in decreased phagocytosis, although inhibition was less marked than that effected by lipopolysaccharide.

Repeated tests revealed that latex particles absorb CA antibodies equally well irrespective as to whether they had been treated with CA alone or with mixtures of CA and lipopolysaccharide or lipoid A, indicating that the latter do not replace CA on the surface of the latex particles. The same results were obtained, also, when latex particles were treated first with CA and then with lipopolysaccharide, or vice versa. Therefore, it may be concluded that the inhibitory effect on opsonization of lipopolysaccharide and of lipoid A is not due to displacement or coverage of CA molecules on the surface of latex particles.

Discussion. Previous experiments have shown that antibodies against the widely distributed common antigen (CA) of enteric bacteria opsonize these microorganisms for phagocytosis by polymorphonuclear leukocytes(3). The present studies have revealed that CA antibodies likewise opsonize antigenically modified latex particles. Unexpected, however, was the observation that latex particles treated with crude supernates of enteric bacteria other than *E. coli* O14, containing both CA and O antigen, were poorly opsonized by CA antibodies, although they were well opsonized by O antibodies. This finding suggested the possibility that lipopolysaccharide interferes with opsonization of latex particles by CA antibodies, and the experiments with highly purified lipopolysaccharide revealed this to be the case. Although less effective, the lipoid A component of lipopolysaccharide also interferes with phagocytosis by CA antibodies. Proof that latex particles take up both antigens and that lipopolysaccharide or lipoid A do not replace CA on the surface of latex particles was obtained in absorption experiments; they revealed that CA antibodies are absorbed equally well by latex particles treated with CA alone as by those treated with CA and lipopolysaccharide mixture. Clearly, latex particles offer distinct advantages over bacteria, for with the former the attachment of antigens in varying amounts can be readily effected. The question remains to be answered why enteric bacteria containing both CA and lipopolysaccharide on the surface are opsonized by CA antibodies. It is conceivable that the relative amounts of the two antigens or their relative location on the surface of the bacterial cells prevents the above described inhibitory effect. It is of more than passing interest that both lipopolysaccharide and its lipoid A component also interfere with the immunogenicity of ethanol soluble CA, for it has been shown that both inhibit the formation of circulating CA antibodies if injected together(9,12). It remains for future investigations to determine the mode of action of these substances as inhibitors of immune phagocytosis by CA antibodies of antigenically modified latex particles.

Summary. Immune phagocytosis experiments were carried out with latex particles modified with common antigen (CA) of enteric bacteria. (1) Latex particles modified with CA (ethanol soluble fraction) of enteric bacteria other than *E. coli* O14 are opsonized in the presence of CA antibodies for phagocytosis by rabbit peritoneal polymorphonuclear leukocytes. (2) CA antibodies also opsonize latex particles treated with the ethanol insoluble CA from *E coli* O14. (3) Latex particles modified with crude supernate of enteric bacteria other than *E. coli* O14 are poorly opsonized by the same CA antisera. (4) Lipopolysaccharide and its lipoid A component interfere with opsonization by CA antibodies. (5) Latex particles modified with both CA (ethanol soluble fraction) and lipopolysaccharide or lipoid A absorb CA antibodies equally well as particles treated with CA alone.

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Molecular Weight Differences in Chicken Isoantibodies.* (30718)

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A number of factors are known to influence the molecular weight of antibodies produced by an immunized animal although several questions regarding the regulation and heterogeneity of the immune response remain unanswered(1). Whether antibodies of high or low molecular weight are formed may depend, in part, on the nature and physical state of the antigen. That this may be particularly true with chickens has been reported(2,3,4). The present study was undertaken to investigate molecular weight differences in chicken isoantibodies specific for 5 blood group systems. Two of the systems are associated with histocompatibility(5). Details of the chicken blood groups may be found in recent reports (6,7).

Materials and methods. Gel filtration. Using Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Rochester, Minn.), the macroglobulins were directly separated from the lower molecular weight γ-globulins of 18 different isoantisera. That the Sephadex method is efficient for fractionating chicken as well as human sera is indicated by several studies(2,4,8,9). The Sephadex column measured 3.5 × 35 cm. Three ml of serum were placed in the column and eluted at room temperature, with a 0.1 M Tris-HCL in 0.2 M NaCl (pH 8) solution. A 0.2 M NaCl (pH 7) solution, used with some of the sera, also proved to be a satisfactory eluant. The eluate was collected in 4 ml portions with a flow rate of approximately 24 ml per hour so that elution was usually completed in 10 hours.

Antisera. The 18 sera were obtained from adult partially inbred chickens that had been hyperimmunized by giving at least 3 intravenous injections of washed erythrocytes at one-week intervals. All of the sera came from birds bled after one or more series of injections. Thus, the antisera were collected from one month to about one year after the first erythrocyte injection. Specificity of the antisera was previously determined by testing with erythrocytes from birds of known blood group genotypes.

Testing the eluate fractions. Two sera, specific for antigens of different blood group systems, were mixed and then passed through the column. Antibody activity of the eluate fractions was determined by agglutination tests. Separate tests were made with erythrocytes from 2 birds differing in blood type for the respective systems, so that each would give a positive reaction with only one antiserum. The tests were made in small tubes by using 0.05 ml of a 2% erythrocyte suspension and 0.1 ml of eluate from each collection tube. The mixture was incubated at

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