from 1-C^{14}-acetate, observed on addition of glucose, indicates that the rate of activation of acetate was accelerated by the breakdown of glucose. It may be assumed that under the experimental conditions employed, the rate of formation of acetoacetyl-coenzyme A from the acetyl-coenzyme A derived from both acetate and glucose may have exceeded the rate of regeneration of reduced pyridine nucleotide coupled with the breakdown of glucose alone. As a result, a large part of the acetoacetyl-coenzyme A must have escaped reduction and have been deacylated.

Summary. 1. The ketogenic power of various C^{14}-labeled substrates, incubated with actively respiring homogenates of mammary gland and of kidney cortex, has been compared by measuring rate of incorporation of the substrate carbon into carrier-acetoacetate. Whereas acetate and pyruvate were strongly ketogenic, glucose and lactate gave rise to only small amounts of acetoacetate. The analogous behavior of glucose and lactate in contrast with that of pyruvate, indicates that the low ketogenicity of glucose and its ability to stimulate lipogenesis is associated with the regeneration of reduced pyridine nucleotides during its breakdown. 2. No antiketogenic action of glucose was observed when this was added to mammary homogenates metabolizing 1-C^{14} acetate. Under certain conditions, the presence of glucose resulted in the acceleration of the appearance of acetate-carbon in respiratory CO_2 and in acetoacetate. This 'ketogenic' action appeared to be due to stimulation by glucose of the activation of acetate and consequently the formation of acetoacetyl-coenzyme A at a rate exceeding the rate of regeneration of reduced pyridine nucleotide coupled with the breakdown of glucose.

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Effect of Trypsin on Agglutinability of Lipopolysaccharide Treated Erythrocytes.*† (23614)

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Crude somatic antigens of various enteric, Gram-negative bacilli as well as their lipopolysaccharide components become readily attached to erythrocytes of man and sheep, resulting in the acquisition of a new serologic specificity. These modified red blood cells thus become specifically agglutinable in the presence of homologous bacterial antibodies. With modified sheep cells lysis occurs upon the addition of antibody and guinea pig complement. It has been shown that erythrocytes from dog, rabbit, guinea pig, rat, and chicken are as readily modified by *Escherichia coli* antigen as human and sheep erythrocytes(1). Similarly, *Salmonella typhosa* Vi antigen be-

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comes attached to red blood cells from man, sheep, horse, ox, goat, and rabbit(2). Recently, a chance observation revealed that alligator erythrocytes after treatment with *E. coli* antigen were not agglutinated upon the addition of the corresponding antiserum. It was decided, therefore, to explore the possibility that enterobacterial hemagglutination does not occur with erythrocytes from certain cold blooded animals. The present study revealed that red blood cells from alligator and axolotl, following treatment with enterobacterial lipopolysaccharides, are not agglutinated by homologous bacterial antibodies and that trypsin treatment of these red blood cells makes enterobacterial hemagglutination possible.

Material and methods. Crude enterobacterial antigens and highly purified *E. coli* lipopolysaccharides were prepared as described previously(3,4). Blood was procured from man (blood group O), alligator (*Alligator mississipiensis*), axolotl (*Ambystoma mexicanum*), snake (*Elaphe o. obsoleta*), and black bass (*Micropterus sp.*). Trypsin (Difco B454) was dissolved as a 1% solution in phosphate hemagglutination buffer (Difco); this stock solution was kept frozen. Trypsin (0.1%) (2 ml) was freshly prepared and added to the sediment of the erythrocyte suspension (2 ml). The mixtures were incubated in a waterbath at 37°C for 15 minutes. Bacterial antiserums were prepared in rabbits; human serums containing enterobacterial antibodies were obtained from patients infected with the particular microorganisms. The enterobacterial hemagglutination and hemolysis tests were carried out according to the methods described previously(3,4), except for the inclusion of trypsin. Phosphate hemagglutination buffer (Difco) was used as diluent unless indicated otherwise.

Results. Treatment of alligator red blood cells with crude enterobacterial antigens obtained from a variety of enteric microorganisms, including *E. coli, Aerobacter aerogenes, Proteus vulgaris,* and *Pseudomonas aeruginosa,* did not result in agglutination upon the addition of homologous bacterial antiserum obtained from either rabbit or man, nor were positive results obtained with *E. coli* lipopolysaccharides. Neither did hemagglutination occur when sodium chloride solutions (0.85% and 0.6% respectively) were used as diluent instead of buffer, or when the experiments were carried out at 22 or 4°C. In contrast, hemagglutination was always demonstrated in parallel experiments with sheep or human erythrocytes. Failure to obtain agglutination with alligator erythrocytes in the above system is not due to inagglutinability of the red blood cells, since an alligator erythrocyte antiserum prepared in rabbits produced agglutination readily. Limited studies on axolotl, snake, and fish cells also yielded the same results.

Subsequently, it was shown that trypsin treatment of both alligator and axolotl erythrocytes made enterobacterial hemagglutination possible. Aliquots of red cell suspensions from 4 animal species were treated with (1) *E. coli* 111 lipopolysaccharide (5 μg/ml), (2) trypsin (0.1%) followed by *E. coli* lipopolysaccharide (5 μg/ml), (3) *E. coli* lipopolysaccharide (5 μg/ml) followed by trypsin (0.1%), and (4) trypsin (0.1%). The cells were washed 3 times before, between, and after treatments with trypsin and/or lipopolysaccharide. The cell suspensions (0.2 ml) were then added to *E. coli* 111 and *S. sonnei* antiserums in various dilutions (0.2 ml). The mixtures were incubated in a waterbath at 37°C for 60 minutes and centrifuged at approximately 1000 rpm. The resulting hemagglutination was observed grossly and is recorded in Table I.

Perusal of the Table reveals that after treatment with *E. coli* lipopolysaccharide neither alligator nor axolotl cells, in contrast to sheep and human erythrocytes, were agglutinated by the homologous antiserum. However, hemagglutination resulted with both alligator and axolotl cell suspensions when the red blood cells were treated with trypsin either before or after exposure to lipopolysaccharide. It is evident also that trypsin treatment alone did not cause non-specific agglutination by *E. coli* antiserum. Important is the observation that trypsin treatment did not substantially increase agglutination of human or sheep red blood cells, either by producing stronger clumping or agglutination in higher

TABLE I. Effect of Trypsin on Agglutinability of Lipopolysaccharide Treated Erythrocytes.

Antiserum		Hemagglutination: Treatment of erythrocytes: LP A	LP B	LP C	LP D	Trypsin-LP A	Trypsin-LP B	Trypsin-LP C	Trypsin-LP D	LP-trypsin A	LP-trypsin B	LP-trypsin C	LP-trypsin D	Trypsin A	Trypsin B	Trypsin C	Trypsin D
E. coli 111	1: 100	—	—	4	4	3	3	4	4	3	3	3	4	—	—	—	—
	1: 200	—	—	4	4	3	3	4	4	3	3	3	4	—	—	—	—
	1: 400	—	—	4	3	3	3	4	4	3	3	3	4	—	—	—	—
	1: 800	—	—	3	3	3	3	3	3	3	3	3	3	—	—	—	—
	1: 1600	—	—	2	1	3	2	3	3	1	1	1	1	—	—	—	—
	1: 3200	—	—	±	—	2	1	—	2	1	—	—	—	—	—	—	—
	1: 6400	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	1:12800	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
S. sonnei	1: 100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	1: 1000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

LP = *E. coli* 111 lipopolysaccharide; A = alligator erythrocytes; B = axolotl erythrocytes; C = sheep erythrocytes; D = human (blood group O) erythrocytes; — = no hemagglutination; 1 to 4 = various degrees of hemagglutination.

serum dilutions. Finally, the specificity of the reactions is evident from the fact that a potent *S. sonnei* antiserum did not cause agglutination of any of the erythrocyte suspensions. Additional control antiserums (*E. coli* 55 and 26), not included in the Table, also failed to produce hemagglutination.

Discussion. Enterobacterial hemagglutination takes place in two stages: in the first phase the enterobacterial antigen becomes attached to the surface of the red blood cells without causing visible changes; and in the second phase agglutination ensues upon the addition of homologous bacterial antibodies. The data reported in this communication clearly show that red blood cells from several cold-blooded animal species treated with these antigens are not agglutinated by the corresponding antiserums. This finding is in striking contrast to the results obtained with erythrocytes from sheep and man. The negative findings obtained with alligator and axolotl cells may be due to either failure of lipopolysaccharide attachment or to interference with the agglutination reaction *per se*. It is the second mechanism which accounts for the observed results, since trypsin treatment results in specific hemagglutination after exposure of the red blood cells to the lipopolysaccharide, as documented in Table I. It is conceivable that the receptors for these antigens of alligator and axolotl red blood cells are located more deeply than those of sheep and human erythrocytes, perhaps like valleys surrounded by walls of protein or protein-like material, or that a protein moiety located between 2 polysaccharide molecules interferes with the spacial orientation of the antibody necessary for agglutination. Removal of the protein or part thereof may make agglutination by antibody of antigen-modified cells possible. The former concept appears to be in agreement with ideas expressed by Coombs and associates(5,6) who studied "inagglutinable" bovine red blood cells and demonstrated that agglutination could be effected by building out a chain of anti-globulin and globulin molecules from the globulin of the antibody sensitizing the cells. Regarding the second possibility, attention may be called to the interference with bactericidal action of O antibodies by R antigen-antibody complex located on the same cell(7).

Additional experiments are needed to determine whether erythrocytes from other cold-blooded animals resemble alligator or sheep cells in the enterobacterial hemagglutination test. Also, similar studies should be carried out with the numerous microbial polysaccharide antigens which are operative in this reaction. For a list of these bacterial antigens the reader is referred to a recent review(8). In particular, the minimal number of molecules of various lipopolysaccharides per red blood cell from different animal species necessary for hemagglutination should be determined. It should be emphasized that marked differences exist between various polysaccharide

antigens of bacterial, fungal, and protozoal origin regarding their suitability for red blood cell modification. For instance, one of the antigens prepared by MacPherson and coworkers(9) did not modify red blood cells and the others became effective only after treatment with NaOH. Enhancement of erythrocyte modifying capacity by heat and sodium hydroxide differs markedly in degree with various salmonella lipopolysaccharides(4). Vogel(10) showed that the erythrocyte sensitizing antigen from *Saccharomyces cerevisiae* is more heat labile than that of *C. albicans*. The suggested investigations may yield information on the identity or lack of identity of receptors of red blood cells from various animal species for different antigens and conceivably on the taxonomic positions of the animals.

Boyden(11) reported that ox red blood cells adsorb mallein without being agglutinated by homologous antiserum. It will be interesting to determine whether trypsin treatment of these erythrocytes makes mallein hemagglutination possible. Furthermore, Boyden noted that different amounts of this antigen were required for modification of erythrocytes from various animal species. In agreement with the above hypothesis it is conceivable that with erythrocytes from certain animal species some of the antigen and the corresponding antibody become attached to sites not available for the hemagglutination reaction. The effect of trypsin treatment of these red blood cells on the minimal amounts of antigen required for hemagglutination should be determined.

It has been known for several years that trypsin treatment of erythrocytes, by an as yet incompletely understood mechanism, makes possible the detection of incomplete Rh antibodies(12,13,14,15). Raffel(16) postulated that the Rh antigen is located deeply, that trypsin removes stromal constituents which hinder contact of the fixed antibody with a second cell, and that the combining groups of "incomplete" antibodies are closer together than those of "complete" antibodies. In the system of lipopolysaccharide modified trypsinated red blood cells, reported here, the reaction takes place between a "complete" antibody and an artificially attached antigen. It is not known whether the same mechanism of trypsin action is responsible for both phenomena. A comparative study on the effects of various proteolytic enzymes, including papain and ficin, in the two systems may yield the answer to this question. It may be mentioned that, had experiments been carried out solely with alligator and axolotl cells, hemagglutination conditioned by trypsin erroneously could have been ascribed to the presence of "incomplete" antibodies. This example serves again as an illustration of the difficulties encountered in a precise definition of "incomplete" antibodies.

Summary. A study on hemagglutination of red blood cells from alligator, axolotl, snake, fish, sheep, and man modified by crude enterobacterial antigens and *E. coli* lipopolysaccharide (5 μg/ml) revealed the following results. (1) Hemagglutination of antigen-modified erythrocytes from the above cold-blooded animals does not take place in the presence of homologous antibodies, either at 37, 22 or 4°C. (2) Under identical conditions hemagglutination is readily demonstrated with human and sheep red blood cells. (3) Trypsin treatment of alligator and axolotl cells either before or after modification results in hemagglutination upon addition of homologous bacterial antibodies. (4) It is concluded that trypsin removes an inhibitor interfering with the agglutination reaction *per se* and not with the attachment of the antigens to the red blood cells of alligator and axolotl.

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Effects of Air Ions on Isolated Rabbit Trachea.* (23615)

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Evidence has recently accumulated indicating that negative air ions are beneficial in certain cases of hay fever and asthma(2). Positive ions, on the other hand, are reported to produce nasal obstruction, dryness of the mucous membranes, and headaches(6,8). In order to test whether these clinical observations could be correlated with any measurable changes in the pulmonary clearing mechanism (including ciliary rate, rate of mucus flow, and smooth muscle tone), the following *in vitro* experiments were undertaken.

Methods. Pieces of fresh rabbit† trachea 2-3 cm long were cut along the anterior wall, spread open, and pinned to small blocks of wood. These blocks were then placed in glass-and-plastic chambers (Fig. 1) containing air of 80-100% relative humidity. By means of a 10X dissecting microscope and a Strobotac-Strobolux combination it was possible to observe the surface of the tissue and determine the ciliary rate ± 50 beats/min. Rate of mucus flow was determined by timing the progress of air-bubbles or of added grains of talc (averaging .01 mm in diameter) in the mucous layer. Clearing efficiency was gaged by the ability of a tissue to remove talc grains applied evenly over its surface with a medical powder blower. The experiments were conducted at room temperature, which varied from 21-23°C. Air ions were generated by beta radiation derived from tritium which was contained in sealed foils; a reversible rectifying circuit made it possible to select positive or negative air ions at will(5). Measurements made with the Beckett probe and Beckman micro-microammeter(3) established that 1 x 10^9 air ions of either charge impinged on each cm^2 of exposed tissue/sec.

Results. 1. *Effect on Ciliary Rate.* Under conditions described above, the initial ciliary rate averaged between 1400 and 1500 beats/min. This level was maintained for a few hours, then slowly declined. In most experiments ciliary activity ceased within 6 hours, although in a few cases it continued for 24 hours. The manner in which rabbits were

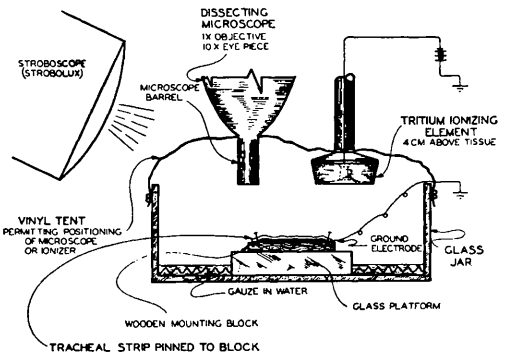

FIG. 1. Arrangement for observing effect of air ions on isolated rabbit trachea. Three such units were available.

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