compared with maternal plasma. Both ethanolamine and choline plasmalogens were present, their relative proportions resembling blood cells rather than plasma. Sphingomyelin and neutral lipid were also present. The PAS reaction (Fig. 4) visualized a number of additional substances, some of which were presumably glycolipid and inositide; the large PAS component near the solvent front resembled monoglyceride. This component was separable into 2 spots on chromatography in n-heptane: 2,6-dimethyl-4-heptanone 100:10 on unimpregnated Whatman No. 1 filter paper. Comparison with authentic samples of monopalmitin and batyl alcohol (α-glyceryl ether) (Fig. 5), showed very close similarities. The lecithin was proportionally much greater than in plasma and did not stain with OsO_4; it further resembled hydrolecithin in its hydrophobic properties.

Discussion. The marked reduction of ethanolamine phosphatide in cord plasma suggests that there may be an excessive demand by the fetus for this lipid. Indeed, in view of the recent demonstration(4) that the biosynthesis of lecithin can be mediated *via* the mono- and dimethyl derivatives of phosphatidyl ethanolamine (and perhaps the plasmalogen analogs as well), these observations are especially interesting.

To the best of our knowledge free glyceryl ethers have been observed on only one other occasion(5), although they have been isolated from the non-saponifiable material from bone marrow(6) and spleen(7) where they were presumably ester-linked to some other lipid component.

Although the phosphatide pattern of amniotic fluid showed a strong resemblance to plasma, the several marked differences imply that it most probably has a mixed origin; the monoglycerides, glycolipids and possibly the hydrolecithin may be contributions by the fetus or amniotic epithelium.

Summary. A paper chromatographic analysis of the lipids in maternal and cord blood and human amniotic fluid was made. The reduced lipid content of cord blood was confined to the plasma where phosphatidyl ethanolamine and the corresponding plasmalogen were disproportionately reduced. The amniotic fluid had a number of PAS positive components not observed in blood cells or plasma.

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Immune Agglutination and Lysis of Antigen Modified Erythrocytes from Various Animal Species.* (27435)

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During the past 15 years it has been shown that many bacterial polysaccharide antigens readily become attached to the surface of red blood cells. Erythrocytes thus endowed with

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a newly acquired antigenic specificity are agglutinated by homologous bacterial antibodies and are referred to, for the sake of brevity, as modified erythrocytes(1). This applies to red blood cells from many animal species, including man, rabbit, sheep, and chicken. In contrast, erythrocytes from certain other species, such as alligator and axolotl, treated with the identical antigens are not agglutinated by the corresponding antibodies. Treatment of these erythrocytes with certain enzymes, such as trypsin and pancreatic protease, however, makes agglutination possible(2,3). The enzymes are equally effective before and after antigen treatment, indicating that they affect the agglutination reaction rather than antigen attachment. Boyden(4) observed that horse erythrocytes treated with mallein were agglutinated by mallein antibodies, but erythrocytes of ox and pig sensitized under the same conditions were not agglutinated. Fujita *et al.*(5) reported that goat erythrocytes modified by *Proteus* OX19 antigen were not agglutinated by the corresponding antibodies, but the *Proteus* OXK antigen-antibody system yielded positive results. Of particular interest is their observation that, in presence of complement, lysis of goat cells occurred with both OX19 and OXK systems. The present investigation revealed striking differences in immune agglutination and hemolysis by enterobacterial O and Vi antibodies of antigen modified erythrocytes from various animal species.

Materials and methods. Antigens were prepared as follows. Smooth strains of *Shigella sonnei,* various salmonellae, and *Staphylococcus aureus* were grown on brain veal agar in Kolle flasks for 18 hours at 37°C. The resulting growth was suspended in phosphate buffer (pH 7.3, Difco) and heated in boiling water for 1 hour. The supernates obtained by centrifugation at 23,500 *g* for 30 min were used as crude antigens in a dilution of 1:10. Dehydrated *S. typhosa* O antigen, prepared by one of us (V.B.), was dissolved in physiological saline solution (10,000 μg/ml); to enhance erythrocyte affinity it was treated with 0.25N NaOH for 30 min at 56°C, and the solution neutralized with 0.25N HCl. Vi (Ballerup) antigen, as employed by Schubert, Edwards, and Ramsey(6), was kindly supplied by Dr. Albert H. Harris, Division of Laboratories and Research, Albany, N. Y., and Vi antiserum by Dr. Philip R. Edwards, Communicable Disease Center, Atlanta, Ga. The other antisera employed were prepared in rabbits in this laboratory.

Human red blood cells (blood group O) were obtained from healthy blood donors; sheep blood from a slaughter house; alligator blood by cardiac puncture from animals at the Buffalo Zoological Garden; ox and goat blood was purchased from Colorado Serum Co., Denver, Colo., and ox blood also from Cappel Laboratories, West Chester, Pa.

The hemagglutination tests were carried out as described previously(2,3). Briefly, the blood specimens were adjusted to hematocrit values of 40 to 43; a 2.5% suspension was washed 3 times, treated with antigen for 30 min at 37°C, and again washed 3 times to remove excess antigen. Antiserum in serial 2-fold dilutions (0.2 ml) was mixed with equal volumes of antigen-modified red blood cells. The mixtures were incubated for 30 min at 37°C, and the resulting hemagglutination was read grossly after centrifugation at 800 *g* for 3 min.

In some experiments red blood cells were treated with both antigen and enzymes. Trypsin was obtained from Difco Laboratories, Detroit, Mich.; pancreatic protease, ficin (fig tree latex), papain (papaya), proteinase (of fungal origin), peptidase (from hog intestinal mucosa) from Nutritional Biochemicals Corp., Cleveland, Ohio; and bromelin from Takamine Laboratories, Clifton, N. J.

For the hemolysis tests antiserum in serial dilutions (0.2 ml) was mixed with equal volumes of antigen modified erythrocytes and with 0.1 ml of 1:10 diluted guinea pig complement (Carworth, Inc., New City, N. Y.). Hemolysis was read after incubation at 37°C for 1 hour.

Latex agglutination tests were carried out as follows. Latex suspension (0.81, Difco) was diluted 1:50 in phosphate buffer and added to equal amounts of antigen solution. The mixtures were incubated at 37°C for 30 min. To 0.5 ml of this reagent, antiserum in

TABLE I. Typhoid O and Vi Hemagglutination and Hemolysis.

Antigen-antibody systems and tests		Erythrocytes: Man	Sheep	Goat	Ox	Alligator
		Antibody titers (reciprocal)				
Typhoid O	Hemagglutination	3200	1600	1600	200	<100
	Protease hemagglutination	6400	3200	3200	12800	800
	Hemolysis	<50	1600	1600	800	< 50
Vi	Hemagglutination	3200	12800	6400	3200	3200
	Protease hemagglutination	6400	12800	6400	25600	6400
	Hemolysis	<200	3200	1600	1600	<200

serial dilutions (0.5 ml) was added. The mixtures were incubated at 37°C for 90 min and centrifuged at 800 *g* for 3 min. The resulting agglutination was read grossly.

Throughout the experiments phosphate buffer (pH 7.3, Difco) was used.

Results. Hemagglutination and hemolysis tests were carried out with erythrocytes from various animal species, employing 2 antigen-antibody systems. The red blood cells were treated with either typhoid O antigen (20 μg/ml) or with Vi antigen (1:200). Erythrocytes were also treated with mixtures of either of the 2 antigens and pancreatic protease (62 μg/ml). The results of the hemagglutination, protease hemagglutination, and hemolysis tests with the corresponding O and Vi antisera are recorded in Table I.

The Table shows that marked differences exist in the results of the hemagglutination and hemolysis reactions, depending upon both the source of erythrocytes and the particular antigen-antibody system. Red blood cells from man, sheep, and goat modified with O antigen are agglutinated by homologous antiserum in high titer; ox erythrocytes in low titer; and alligator erythrocytes not at all. Protease treatment of ox and alligator erythrocytes markedly enhances agglutinability, whereas this enzyme has but little effect on erythrocytes from man, sheep, and goat. In contrast to O antibodies, Vi antiserum in high dilution agglutinates antigen-modified erythrocytes from all animal species, even without protease treatment. The hemolytic modification of the hemagglutination reaction reveals still further differences between antigen-modified erythrocytes from various animal species, for neither O nor Vi antibodies and complement cause lysis of human and alligator erythrocytes, in contrast to those from sheep, goat, and ox.

Additional hemagglutination and hemolysis studies with O antigens of *S. sonnei* and *S. enteritidis* as well as heterogenetic antigen of staphylococci(7) revealed results similar to those obtained with typhoid O antigen.

Protease treatment of ox erythrocytes, both prior and subsequent to modification with O antigens, enhances agglutinability, indicating that this enzyme does not merely make possible antigen attachment. Additional experiments with other enzymes (250 μg/ml) revealed that trypsin, bromelin, and ficin markedly enhance the hemagglutination reaction of ox erythrocytes treated with O antigens, and that papain, proteinase, and peptidase are only minimally effective.

The observation that ox and alligator erythrocytes modified by Vi antigen are well agglutinated by Vi antiserum suggests the possibility that Vi antigen may have a nonspecific enhancing effect on agglutinability. If this were so, then, ox and alligator red blood cells modified with both antigens should be agglutinated by each of the corresponding antibodies. Repeated experiments, however, revealed that simultaneous or consecutive treatment of these erythrocytes with O and Vi antigens does not result in enhanced agglutination by O antibodies.

It is well known that certain bacteria containing 2 antigens are not agglutinated equally well by both antibodies. Vi antigen in typhoid bacilli interferes with agglutination by O antibodies. Similarly, as shown by Spaun(8) and corroborated by Ceppellini and Landy(9,10), Vi antigen also interferes with agglutination by O antibody of red blood cells modified with both antigens. In the present

TABLE II. Effect of Vi Antigen on Agglutination of Latex Particles and Erythrocytes in Presence of Typhoid O Antigen and Antibodies.

	Latex particles			Erythrocytes		
	Antigens					
	Vi	O	Vi+O	Vi	O	Vi+O
Antisera	Antibody titers (reciprocal)					
O	<50	800	<50	<50	1600	200
Vi	400	<200	400	1600	<200	1600

study the inhibitory effect of Vi antigen on O hemagglutination was demonstrated with human, sheep, ox, and alligator erythrocytes. With red blood cells from the latter 2 species enzyme treated erythrocytes had to be used. The question arises whether Vi antigen displaces O antigen on the surface of the erythrocytes and/or inhibits the agglutination reaction. Therefore, absorption tests were carried out. Human and ox erythrocytes were treated with typhoid O and/or Vi antigens and used for absorption of typhoid O antiserum. Titration of the unabsorbed and absorbed antiserum revealed that Vi antigen does not decrease the O antibody absorbing capacity of erythrocytes coated with both antigens.

Finally, experiments were undertaken to determine whether Vi antigen also inhibits agglutination by O antiserum of latex particles coated with both antigens. The results of a representative experiment are recorded in Table II and show that the simultaneous presence of Vi and O antigens markedly decreases the agglutinability of both latex particles and erythrocytes by typhoid O antiserum.

Discussion. The present investigation has revealed that agglutination by bacterial antibodies of antigen modified erythrocytes depends upon several factors, notably the antigen-antibody system and the species source of the red blood cells. Erythrocytes from man, sheep, and goat, modified by O antigens from salmonellae or *S. sonnei*, are agglutinated by the homologous antisera in high titer. In contrast, similarly treated ox erythrocytes are agglutinated only in low titer, and alligator red blood cells not at all. It was surprising to learn, therefore, that Vi antiserum in high titer readily agglutinates Vi-modified red blood cells from all these animal species. The fact that certain proteolytic enzymes, such as pancreatic protease, renders O antigen modified erythrocytes from ox and alligator agglutinable by the homologous antisera in high titer strongly suggests that poor agglutinability is not due to failure of antigen attachment. The differences in agglutinability of erythrocytes from various species, noted in the present study, between O and Vi antigen-antibody systems are similar to those observed by Fujita *et al.*(5) with 2 *Proteus* antigen-antibody systems. The question arises, therefore, whether O and Vi antigens become attached at different sites of the erythrocyte from certain species. It is conceivable that the poor agglutinability of O antigen modified ox and alligator erythrocytes by homologous antibodies is due to some anatomic peculiarity of the erythrocyte, whereby the antibody attached to the antigen does not protrude sufficiently to interact with antigen attached to another red blood cell. Proteolytic enzymes may remove parts of the surface material impeding interaction between red blood cells. Differences in length or configuration of antigen and antibody molecules may also play a role.

The observation(8,9) of the inhibitory effect of Vi antigen on agglutination by O antibodies of red blood cells modified by the 2 antigens has been confirmed and extended. With enzyme treated erythrocytes it was shown that Vi antigen inhibits O agglutination of ox and alligator cells as well. Similarly, latex particles coated with both antigens are agglutinated significantly less by O antibodies than particles coated with O antigen alone. In spite of this marked agglutination inhibitory effect of Vi antigen it was observed that erythrocytes modified with both O and Vi antigens effectively absorb O antibodies. The mechanism whereby Vi antigen inhibits agglutination of certain bacteria, antigen modified red blood cells, and antigen coated latex particles remains to be elucidated.

Summary. Study of agglutination and lysis of antigen-modified erythrocytes of various animal species by corresponding antibodies revealed the following results. (1)

Erythrocytes from man, sheep, and goat, modified by O antigens from enterobacteriaceae and heterogenetic (staphylococcal) antigen, are readily agglutinated by the corresponding antibodies, and treatment of these erythrocytes with proteolytic enzymes only slightly enhances the agglutination reaction. (2) In contrast, similarly treated erythrocytes from alligator and ox are not agglutinated or agglutinated only in low titer; enzyme treatment markedly enhances the agglutination reaction. (3) Erythrocytes from all these animal species modified by Vi antigen are readily agglutinated by Vi antiserum in high titer. (4) Poorly agglutinable ox erythrocytes modified by O antigen are lysed in the presence of O antibody and guinea pig complement. (5) Vi antigen present together with O antigen on latex particles and erythrocytes significantly inhibits agglutination by O antibodies. (6) Erythrocytes modified by both O and Vi antigens effectively remove O antibodies.

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Effect of Adrenalectomy and Adrenocorticoid Replacement on Lactational Performance in Mice.*† (27436)

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The necessity of adrenocorticoids for maintenance of lactation has been demonstrated in rats(1,2,3), guinea pigs(4,5), and dogs (6). Studies by Gaunt(7,8,9) and Cowie (10,11,12,13) have been directed toward determining levels of various adrenocorticoids required for maintenance of normal lactation in adrenalectomized rats. A similar approach in determining adrenocortical requirements for lactation in mice has been lacking. This study was undertaken to determine levels of hydrocortisone and desoxycorticosterone required to support normal lactation in mice.

Materials and methods. Albino mice of Webster-Swiss strain were maintained on Purina lab chow pellets in an animal room with constant temperature of 78 ± 1°F. Females were bred in community pens with 6 females to one male per pen. When a female was observed to be in late pregnancy she was transferred to an individual litter cage. With the exception of normal control group, dams were adrenalectomized on day 4 of lactation and litter was reduced to 6 pups. In addition to a normal control group various treatments after adrenalectomy included 7 experimental groups as listed in Table I. Carrier consisted of 5 mg gum arabic per 100 mg adrenocorticosteroid brought to total volume with physiological saline.

Criteria of lactational performance included litter weights on days 4, 14, and 20 of lactation, mortality rates of pups on days 14 and 20 of lactation and in Groups a, g, and h

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