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Properties of Guinea Pig 7S Antibodies. VI. Transmission of Antibodies From Maternal to Fetal Circulation.* (28591)

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Evidence provided by several investigators (1-4) suggests that in some animal species fetal membranes select certain maternal antibodies for transmission to the fetal circulation and exclude other antibody fractions. For example, in the human, $7S\gamma_2$ antibodies reach the fetal circulation, but γ_{1A} (β_{2A}) and γ_{1M} (β_{2M}) antibodies appear to be excluded (1). In the rabbit and guinea pig, fetal membranes exposed to homologous and heterologous tetanus or diphtheria antitoxin transmitted the homologous antibody more readily than the heterologous antibody(2-4). Evidence has been presented that the process of selective transmission involves a property of piece III, obtained by papain digestion of 7S γ-globulin(5). In contrast to the significant transmission of natural diphtheria antitoxin, pepsin-digested antitoxin, lacking a major part of piece III, was transmitted to the guinea pig and rabbit fetus in only trace amounts(2, 6). Conversely, fragment III of rabbit γ-globulin was transmitted nearly as readily as whole γ-globulin, whereas fragments II and I were transmitted only 1/5 and 1/10 as readily as fragment III(5).

Recent studies have characterized 2 types of guinea pig 7S antibody capable of reacting specifically with the same antigen but differing in electrophoretic mobility and biological properties(7,8,9). It was shown that 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 $7S\gamma_1$, antibodies fixed complement *in vitro* in the presence of antigen under the conditions tested and sensitized antigen-coated erythrocytes for lysis in the presence of complement. According to current concepts of the structure and function of mammalian γ-globulins, differences between guinea pig $7S\gamma_1$ and $7S\gamma_2$ reside in the piece III (F fragment) of the antibody molecule(7). The availability of these 2 types of 7S antibodies led us to test (a) whether both antibodies were transferred through fetal membranes and (b) whether biologic properties associated with only one or the other of the antibodies would favor transmission or rejection by fetal membranes.

In the guinea pig, antibody is transferred from the maternal circulation into the uterine lumen and across the visceral endoderm of the yolk sac into the vitelline vessels(4). Later in pregnancy and early during the postnatal period, certain antibodies may be transferred via the fetal gut(10-12).

Methods. Animals: Hartley-strain, random-bred guinea pigs were used for immunization and for active and passive antibody transfer studies.

Conjugated antigens: Bovine gamma globulin and bovine plasma albumin (Armour Pharmaceutical Co., Kankakee, Ill.) were highly conjugated with 2,4-dinitrofluorobenzene (Eastman Organic Chemicals, Rochester, N. Y.) as described (7).

Method of immunization and assay of antibody activities: The schedule of immunization

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TABLE I. Transfer Across Fetal Membranes of γ_1 and γ_2 Guinea Pig Antibodies in Actively Immunized Animals.

Group	Duration of maternal immunization (days)		Passive hemagglutination	Passive hemolysis	C.F.	P.C.A.
	Prior to pregnancy*	Total	Function of			
			$\gamma_1 + \gamma_2$	γ_2	γ_2	γ_1
Mother I		20	2560†	1280†	80†	2000†
Offspring Ia			640	320	20	1000
" Ib			1280	640	40	1000
" Ic			640	640	40	1000
Mother II	37	107	2560	640	40	5000
Offspring IIa			2560	640	20	5000
" IIb			2560	320	5	5000
" IIc			2560	640	10	5000
" IId			2560	640	20	5000
Mother III	40	110	5120	2560	80	5000
Offspring IIIa			5120	1280	40	3000
Mother IV‡	19	89	640	0	0	2000
Offspring IVa			640	0	0	1500
" IVb			640	0	0	1000

* Approximate duration based on gestation period of 65-70 days(10).
† Reciprocal of titer.
‡ Immunized intraperitoneally without adjuvants.

used to prepare antisera for passive transfer was previously described(7). The technique of passive cutaneous anaphylaxis (PCA), complement fixation (CF), passive hemagglutination and hemolysis of hapten-conjugate-coated, tannic-acid-treated sheep erythrocytes were performed as described (8,9).

Active immunization of pregnant guinea pigs: Female guinea pigs were immunized with 1 mg 2,4-dinitrophenyl bovine gamma globulin (DNP-BGG) in complete Freund's adjuvant; this mixture was distributed in 0.25 ml aliquot in each footpad. Guinea pig No. 4 was injected intraperitoneally with 1 mg DNP-BGG in saline daily for 3 days. Beginning one week later, and continuing throughout pregnancy, all animals were injected with 100 μg DNP-BGG in 0.1 ml saline at 4 intradermal sites. Following initial immunization, guinea pigs were mated, and became pregnant 2½ to 5½ weeks later. Guinea pig No. 1 was immunized with adjuvants late in pregnancy.

Newborn guinea pigs were removed from the mother prior to suckling and were exsanguinated under nembutal® anesthesia via the carotid arteries. Postpartum guinea pigs were bled at the same time from the retro-orbital venous plexus. Guinea pig milk was obtained by gentle pressure on the mammary gland.

Passive immunization of pregnant guinea pigs: Late-pregnancy guinea pigs were injected intravenously with 1.5 to 2.0 ml aliquots of high-titer guinea pig anti-DNP-BGG antisera at intervals of 2 hours (for total dose see Table II). Forty-eight hours after the final injection, fetuses were delivered by Cesarean section performed under ether anesthesia. Fetuses and mother guinea pigs were exsanguinated as above.

Results. Table I summarizes the results of tests performed on serum from hyperimmunized female guinea pigs and their offspring. The maternal:newborn ratio of antibody titer in Groups I to IV was approximately 1:1 to 1:2. This ratio obtained for tests involving functions of guinea pig $7S\gamma_2$ antibodies (passive hemolysis and complement fixation) as well as for tests involving a function of guinea pig $7S\gamma_1$ antibodies (passive cutaneous anaphylaxis). Furthermore, within a group, the ratio of γ_1 to γ_2 antibodies in maternal serum (as determined in the above tests) was similar to the ratio of γ_1 to γ_2 antibodies in the serum of her offspring.

Guinea pig IV, immunized before pregnancy with DNP-BGG administered intraperitoneally without adjuvants, produced moderate amounts of $7S\gamma_1$ antibodies(7). Gamma-2 antibodies could not be detected by passive

TABLE II. Transfer Across Fetal Membranes of γ_1 and γ_2 Guinea Pig Antibodies in Passively Sensitized Animals.

Group	Dose of donor serum admin., ml	Wt of pregnant guinea pig, g	Passive hemagglutination	Passive hemolysis	C.F.	P.C.A.
			Function of			
			$\gamma_1 + \gamma_2$	γ_2	γ_2	γ_1
Mother V	5.2	1250	320*	80*	2.5*	100*
Fetus Va			20	10	0	40
" Vb			40	20	0	60
" Vc			40	20	2.5	40
Donor #60			20,480	5120	80	6000
Mother VI	10	1400	320	640	20	600
Fetus VIa			160	80	5	300
" VIb			80	80	5	200
" VIc			80	80	—	300
Donor #63			10,240	10,240	80	5500
Mother VII	12	1330	640	160	20	200
Fetus VIIa			80	40	2.5	40
" VIIb			160	40	2.5	40
" VIIc			80	40	2.5	40
Donor #64			10,240	5120	80	5500

* Reciprocal of titer.

hemolysis or complement fixation tests in the serum of the mother or her offspring.

Table II summarizes results of tests performed on maternal and fetal serum obtained 48 hours after intravenous administration of donor serum containing anti-hapten antibodies. These tests indicate that passively administered guinea pig $7S\gamma_1$ and $7S\gamma_2$ antibodies reached the fetal circulation. The maternal:fetal ratio of antibody titer in Groups V to VII varied from approximately 4:1 to 8:1 in tests reflecting activity of γ_2 antibodies and varied from 2:1 to 5:1 in PCA. However, more precise methods are required to establish significant differences, if any, in the readiness with which the two types of antibodies reach the fetal circulation.

Table III compares the titers of anti-hapten antibodies detected by PCA tests performed on simultaneously collected specimens of milk and serum. Low titers of gamma-1 antibodies were detected in milk compared to the moderate to high titers simultaneously present in serum. The marked anti-complementary effect of milk interfered with the assay for gamma-2 anti-hapten antibodies by complement fixation or hemolysis. However, both gamma-1 and gamma-2 globulins were detected on immunoelectrophoresis of guinea pig milk using rabbit anti-guinea pig γ_1 and anti-γ_2 antisera to develop the precipitin arcs.

Discussion. The experimental evidence indicates that guinea pig $7S\gamma_2$ and $7S\gamma_1$ anti-hapten antibodies present in the serum of actively or passively immunized pregnant guinea pigs were both transmitted to the young in significant amounts. The presence or absence of a site or configuration involved in complement fixation (presumably present on piece III of guinea pig $7S\gamma_2$) or species-specific skin fixation (presumably present on piece III of guinea pig $7S\gamma_1$) did not appear to be critical for the selection of either antibody for transmission accross fetal membranes; nevertheless, it is probable that a property of piece III of guinea pig γ_1 and γ_2 antibodies is involved in fetal transfer. Our experiments were not designed to test small differences in the relative rate of transfer of γ_1 and γ_2 antibodies. Apparent differences in transfer of γ_1 compared to γ_2 antibodies in animals in Groups V and VI may only reflect

TABLE III. Comparison of Titers of Skin-Sensitizing Guinea Pig Antibodies in Milk and Serum.

Source of milk and serum	PCA titer	
	Milk	Serum
Mother I	50*	2000*
" II	300	5000
" IV	20	2000

* Reciprocal of titer.

differences in sensitivity of the assay methods used.

Guinea pig skin-sensitizing γ_1 antibodies were readily detected in milk from actively immunized mothers. However, it remains to be determined whether antibodies reached the milk by specific transport across acinar structures or by simple transudation.

Present experiments confirm earlier studies (reviewed in 13) on fetal transmission of anaphylactic sensitivity in the guinea pig and underscore an additional major difference between guinea pig and human skin-sensitizing antibodies. Although antibodies with tissue-sensitizing properties in guinea pig and man (14) migrate among the fast moving immune globulins on electrophoresis, they differ markedly in other physical properties. Guinea pig skin-sensitizing antibodies are produced in comparatively large amounts, they belong to the 7S group of globulins(7), and in our experiments are resistant to heating at 56°C for 4 hours and to dissociation with mercaptoethanol. The sedimentation characteristics of human skin-sensitizing antibodies are currently being reinvestigated; in one instance, a human skin-sensitizing anti-glucagon antibody was recently shown to have an intermediate sedimentation rate in the range of 8-11 S (15). Furthermore, human reagins are destroyed by heating at 56°C for 4 hours(16) and are readily dissociated with sulfhydryl compounds(17). Finally, in marked contrast to guinea pig skin-sensitizing $7S\gamma_1$ antibodies, reagins have been reported not to be transmitted from mother to child(13,18).

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A Respiratory Disease Syndrome in Chickens Fed Essential Fatty Acid Deficient Diets. (28592)

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Linoleic acid and to a lesser extent, arachidonic acid, commonly termed "essential fatty acids," are required nutrients for mammalian species(1). Essential fatty acids are also required for maximal growth by the chick(2,3) and cannot be synthesized *de novo* under ordinary conditions by either the hen or the chick(4,5,6). Feeding essential fatty acid-free diets to rats results in cessation of growth, increased basal metabolic rate and dermatitis. Bieri *et al*(7) fed chicks a fat-free diet for 12 weeks but did not observe extreme