

Impermeability of Pig Placenta for Antibodies

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ABSTRACT. After passive immunization of pregnant swine 24 and 48 hours before delivery with isolated fraction of 7S gamma globulin or with the whole serum of a high titer of antibodies to diphtheria and tetanus toxoid no traces of antibodies were detected in newborn piglets by passive hemagglutination even if the globulin fraction of their sera was many times concentrated.

In a series of experimental works we studied the globulin spectrum of sera of piglets which did not receive maternal colostrum. Only after concentration of serum globulins the presence of a small amount of gamma globulin was detected which had different characteristics from gamma globulin of adult animals (Šterzl *et al.*, 1960a, b). It was distinct in physical-chemical characteristics and in peptide composition of structural subunits (Franěk *et al.*, 1961; Franěk & Říha, 1964). By incorporation of S³⁵ methionine and of a mixture of C¹⁴ amino acids it was found that the protein was of gamma globulin nature, was not passively transferred from the mother to the young but was actively synthesized by the newborns. It did not display antibody activity to any of the bacterial, tissue, phage and viral antigens which were tested (Šterzl *et al.*, 1960a, b; Šterzl *et al.*, 1965). The question of possible transfer even of minute amounts of antibodies from the mother to the young across the six-layer placental barrier in pigs is decisive in interpretation of a series of our experiments using this model. In previous work it was therefore studied whether transfer

of antibodies could be demonstrated after intensive immunization of the mother with bacterial and blood cell antigens. However, any minute amounts of antibodies could not be detected in sera of newborn piglets nor in the concentrated gamma globulin fractions (Šterzl *et al.*, 1960a). In contrast Myers and Segre (1963) refer transfer of low amount of antibodies which are demonstrated only after 10-fold concentration of newborn sera. The difference between our previous experiments and their work is to be seen in the type of antigen and in the way of antibody transfer. In their work animals were immunized with diphtheria and tetanus toxoid. Antibodies to these antigens are represented in hyperimmune sera predominantly by gamma globulins of the 7S type (in contrast to antibodies to bacterial and blood cell antigens which are in hyperimmune animals mostly of the 19S type) so that with respect to their lower molecular weight they can more probably be transferred across placenta. In one of their experiments globulin fraction was prepared from hyperimmune serum and the mother passive-

ly immunized before delivery. In our work the same procedure was used.

MATERIALS AND METHODS

Adult pig weighing 80 kg was immunized with 2 ml of diphtheria toxoid (80 Lf) and tetanus toxoid (60 Lf) solution mixed with 3 ml of incomplete Freund adjuvant. Such adjuvant immunizing dose was injected twice in monthly intervals into the foot pads. After another month the animals were injected with 2 ml of diphtheria and tetanus toxoid solution intravenously and two weeks later were bled out.

Isolation of gamma globulin fraction from hyperimmune serum was done by a combined method of rivanol (Hořejší & Smetana, 1954) and ethanol fraction-

ation (Oncley *et al.*, 1949). Serum was added to a five-fold volume of 0.3% rivanol on intensive stirring and the precipitate thus formed was removed by centrifugation. The clear supernatant was made free of rivanol by adsorption on charcoal and after adjusting pH to 5.15 it was precipitated at -5°C by cold 96% ethanol to the final concentration of 17%. The precipitate thus formed containing the major part of beta globulin fractions was centrifuged off. After adjusting pH to 7.2 the purified 7S gamma globulin was isolated from the supernatant by precipitation with ethanol, to 25% concentration. Gamma globulin prepared in this way was dissolved after freeze-drying, sterilized by filtration and applied in the form of 10% isotonic solution.

Concentration of piglet sera was done

Table 1. Antibody titers to diphtheria (Di) and tetanus (Te) toxoid estimated by passive hemagglutination

		Di toxoid	Te toxoid
Pig immunized with Di + Te toxoid	Serum	1 : 102,400	1 : 25,000
	Gamma globulin fraction	1 : 16,000,000	1 : 1,050,000
Pregnant swine injected with immune serum	Serum before } delivery	1 : 512	1 : 512
	Serum at }	1 : 16,300	1 : 65,500
	Sera of newborns No. 1—3	0, 0, 0	0, 0, 0
	γ -Globulins of newborn sera, concentrated 20 times	0	0
Pregnant swine injected 24 h before delivery with gamma globulin fraction of immune serum	Serum before injection	1 : 8	1 : 8
	Serum at delivery	1 : 256	1 : 32
	Sera of newborn piglets No. 1—10	0, 0, 0, 0, 0 0, 0, 0, 0, 0	0, 0, 0, 0, 0 0, 0, 0, 0, 0
	γ -Globulin fraction of the pooled newborn sera, conc. 50 times	1 : 16	1 : 16
	γ -Globulin fraction after absorption with sheep red blood cells	0	0

as follows: Gamma globulin fraction was first isolated by precipitation with ammonium sulphate, the fraction then dissolved, dialyzed and the resulting solution concentrated by pressure dialysis against saline to 1/50 or 1/20 of the original volume. However, after 50-fold concentration the proteins began to precipitate from the solution.

Antibodies were estimated by passive hemagglutination on adsorption of purified diphtheria and tetanus toxoid to tannic acid treated red blood cells (Stavitsky, 1954). Sensitive detection of antibodies was accomplished by using 0.1% suspension of red blood cells and by microhemagglutination test (Takátsy & Hamar, 1955).

RESULTS

The group of piglets were obtained from a mother injected 24 hours before delivery with 5 g of 7S gamma globulin isolated from serum of immunized pig and dissolved in 50 ml of saline. High titers (Tab. 1) both of native serum and of gamma globulin fraction from immunized pig resulted in an increase of the level of antibodies to diphtheria as well as to tetanus toxoid in the mother within 24 hours. However antibodies were not detected in sera of newborn piglets. The pooled sera of the newborn piglets were fractionated and concentrated; thus, the gamma globulin fraction contained 50 times more proteins as compared to the fraction of native sera. The concentrated gamma globulin fraction had tendency to aggregate and precipitate from the solution. With this fraction, normal sheep erythrocytes and erythrocytes with adsorbed diphtheria and tetanus toxoid agglutinated in the same dilution, 1 : 16. However, when such a concentrated fraction was absorbed with sheep red blood cells only, the agglutinating activity was lost not only against normal erythrocytes but also against erythrocytes with adsorbed antigens; this demonstrates that specific antibodies to diphtheria and tetanus toxoid were not transferred.

For eliminating the possible selection of some antibodies during isolation of gamma globulin, another pregnant swine was injected with complete immune serum of a pig immunized with diphtheria and tetanus toxoid. In this experiment no agglutinating activity was found either in native sera of newborn piglets or in the gamma globulin fraction of these sera concentrated only 20 times (in order to maintain all proteins in solution).

DISCUSSION

Evaluating critically the results of the present work, we did not demonstrate transfer of 7S antibodies to diphtheria and tetanus toxoid which were injected either as gamma globulin fraction or as whole serum. The result is similar to the findings of Myers and Segre (1963) who did not demonstrate serologically the presence of transferred antibodies in native sera of newborns delivered by the mother passively immunized with antitoxin sera. However, these authors demonstrated antibodies in the globulin fraction of piglet sera after 10-fold concentration. In order to explain the fact that without detecting antibodies in native serum the titer of 1 : 1024 was found after 10-fold concentration they assumed that an inhibitor was removed by fractionation of the serum. In our experiments different results were obtained. If concentration of newborn sera did not result in aggregation of the gamma globulin fraction, agglutination of normal erythrocytes or of erythrocytes with adsorbed antigens was not observed (Tab. 1, exp. No. 1). In the second experiment, piglet sera concentrated 50 times exerted non-specific clumping of red blood cells. This may explain the agglutination titers demonstrated after concentration of gamma globulin fraction in the work of Myers and Segre (1963). From the results of these authors it is evident that the concentrated fractions were not absorbed with normal erythrocytes.

The experiments show the importan-

ce of controlling the specificity if anti-gen-antibody-like reaction is observed in precolostral piglet sera (Šterzl *et al.*, 1964).

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