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Detection of passage and absorption of chicken egg yolk immunoglobulins in the gastrointestinal tract of pigs by use of enzyme-linked immunosorbent assay and fluorescent antibody testing

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Summary

Chicken egg yolk IgG can be absorbed and transferred as efficiently as colostral antibodies in the blood of neonatal pigs. Egg yolk IgG has a half-life of 1.85 days in newborn pig serum. This is shorter than the reported half-life (12 to 14 days) of homologous IgG in serum of pigs. Similar to colostral antibodies, egg yolk IgG absorption from intestine ceased at about 34 hours of age, after a logarithmic decrease in absorption rate from birth. Egg yolk IgG absorption inhibition time in the gastrointestinal tract took 1.73 hours to decrease by half. Egg yolk IgG was protective against experimentally induced diarrhea in pigs when it was administered at high dose, and multiple dosing was instituted. Adverse effects were not observed when chicken egg yolk IgG was administered orally to pigs.

Use of chicken egg yolk antibodies for orally administered passive immunization against infectious intestinal diarrheal diseases in animals has gained wide attention in recent years, because of the potential therapeutic value these antibodies offer and the practical methods by which they can be prepared for large-scale application. Evidence of their protective capacity has been reported.[1,2] These antibodies are active against a specific antigen and are as useful for protecting animals from attack by that antigen as for immunization of chickens. To be effective, orally administered antibodies must survive enzymatic breakdown within the stomach and intestine and reach their target areas with their structure and function preserved.

Several studies of the passage of colostral antibodies through the gastrointestinal tract and their absorption into the circulation of neonatal animals have been reported. At birth, pigs obtain immunoglobulins from the colostrum of the dam,[3-5] and these immunoglobulins are absorbed by the small intestinal epithelium and transported to the circulation.[6] This phenomenon has a major role in protection against infections during the neonatal period.[4,7-9] Homologous immunoglobulins from colostrum and milk of sows are referred to as maternal antibodies. These antibodies are the first line of defense in suckling pigs, against exposure to infective agents in the environment. Antibodies are innocuous, naturally acquired, and indigenous to the porcine species. Oral administration of chicken egg yolk antibodies to colostrum-deprived pigs is an artificial means of providing protective antibodies. They are heterologous antibodies: not acquired from a natural source, but from a different animal species and, hence, may be rejected or tolerated by the host.

Whether chicken egg yolk immunoglobulins would have the same fate as colostral antibodies in the gastrointestinal tract of pigs was investigated. The purposes of the study reported here were to determine the kinetics of orally administered chicken egg yolk antibodies as they pass through the gastrointestinal tract of pigs, their absorption and transfer to the circulation, and their activity against a particular antigen in the small intestine, thus clarifying their role in protection. A preliminary report on the efficacy of the orally administered chicken egg yolk antibodies against experimentally induced enteric colibacillosis caused by enterotoxigenic *Escherichia coli* in pigs has been published.[1]

Materials and Methods

Pigs—Ninety-five newborn Large White pigs were used in experiments involving oral administration of antibody preparations. Five-month-old White Leghorn hens (strain Hyline W36) were used for immunization with pili, for development of egg yolk pilus-specific antibodies.

Immunization of chickens—Enterotoxigenic *Escherichia coli* (ETEC) strain 19304 (0157:K88ac:NM)[a] grown in minca broth was used.[10] Cells

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[a] Salsbury Laboratories, Charles City, Iowa.

were harvested by centrifugation and used for preparative extraction of fimbriae. Further purification of K88 fimbriae was done by use of affinity column chromatography as described.[11] Purified fimbrial vaccine,[1] containing 0.5 mg of fimbrial antigen in emulsion oil mixed with 5% mannide monooleate,[b] was injected IM (breast muscle) into the hens. Six weeks after initial inoculation, hens were given booster inoculation in similar manner, and their eggs were harvested 2 weeks later.

Purification of antibodies from chicken egg yolk—The egg yolks were carefully separated from the egg whites and yolk membranes. The separated yolks were diluted with 8 volumes of distilled water. An aqueous suspension of 5% hydroxypropylmethylcellulose phthalate[c] in 80% ethyl alcohol was added to the diluted egg yolk gradually, while mixing evenly; the mixture was kept at 10 C for 18 hours. The supernatant was collected and passed through a 0.45-μm membrane filter, and the IgG fraction was purified by affinity chromatography, using a synthetic ligand gel.[12,d]

Immunologic assay—For detection of egg yolk IgG, an indirect ELISA was performed, using chromatographically purified chicken IgG[e] as reference protein. Indirect ELISA also was performed to detect anti-K88 fimbrial antibody activity, and titer was determined by plotting the optical density values of the samples against a standard curve formed by correlated ELISA optical density values and agglutination titers for several chloroform-extracted egg yolk antibodies.[1]

Indirect fluorescent antibody test—The small intestine of pigs was examined by use of the indirect fluorescent antibody (IFA) method. Tissue specimens were taken from the upper, middle, and lower regions of the small intestine. A 2.5-cm (approx) section of intestine was excised and immersed in cold n-hexane at −80 C overnight. Tissue was supported in gelatin specimen-embedding compound,[f] and transverse sections (6 μm) were cut by use of a cryostat[g] at −20 C. Sections mounted on slides were dried in air, fixed in acetone at −20 C for 10 minutes, and stained. Anti-chicken IgG of rabbit origin[e] was used as the primary antibody, and fluorescein isothiocyanate-conjugated anti-rabbit IgG[e] was used as the secondary antibody. Serum from nonimmunized rabbits was used as the primary antibody for the control specimens.

Experimental protocol—To determine the passage time (experiment 1), absorption and transfer of chicken egg yolk antibodies from the gastrointestinal tract to the circulation (experiment 2), and efficacy of the antibodies against experimentally induced diarrhea in neonatal pigs (experiment 3), the antibodies were orally administered to pigs. Collection of intestinal specimens was done at certain time intervals. In the protection experiment, test pigs were pretreated with egg yolk antibodies prior to challenge exposure with virulent K88+ *E coli*.

Experiment 1: Passage of orally administered purified antibodies in the small intestine of pigs in various age groups and distribution pattern of antibodies in various sections of intestine—A total of 30 Large White pigs were given antibodies at separate feeding schedules—10 hours and 3, 6, 21, and 28 days after birth. The antibody preparation was administered only once at the aforementioned feeding hour, which corresponded to the age of pigs at administration of antibodies. All together, 5 age groups of pigs consisted of 6 test pigs and 1 control pig/group. Mean body weight ranged from 1.3 kg in young pigs to 7.5 kg in older pigs. Collection of blood samples was done separately at 2, 6, and 24 hours after antibody administration, and 2 pigs in each age group were euthanatized immediately after every blood sample collection. Pigs were euthanatized by overdose of pentobarbital sodium[h] and necropsy was immediately done to remove the entire gastrointestinal tract; specimens were collected from various sites. Test pigs each were given 5 ml of the egg yolk IgG solution (anti-K88 fimbrial antibodies; titer 4,800) at dosage of 100 mg/ml, inoculated orally through a 5-ml syringe attached to a length of silicone tube that was held in the pig's mouth. The distribution of antibodies in the gastrointestinal tract was determined by use of the chicken IgG and anti-K88 fimbrial antibody-specific ELISA and IFA examination of the small intestine.

Experiment 2: Absorption of orally administered egg yolk IgG in the blood of various age groups of pigs—Two phases of the experiment were carried out: to determine the approximate cutoff age at which absorption of egg yolk IgG in the blood is observed and the half-life of absorbed IgG; and to account for the decrease in absorption rate of chicken IgG within the age groups of pigs at which absorption occurs.

For phase 1, a total of 10 Large White pigs were fed antibodies separately at 10, 34, 58, and 82 hours after birth. The various feeding hours corresponded to the age groups of pigs that were given the antibody preparation. The 10-hour age group consisted of 4 pigs with mean body weight of 1.4 kg, whereas the 34-, 58-, and 82-hour age groups consisted of 2 pigs each, with body weight ranging from 1.6 to 2.0 kg. Blood sample collection was done sequentially in all the groups at 2 hours and at 2, 4, 6, 14, 21, and 28 days after antibody administration. The pigs each were given 5 ml of the egg yolk IgG solution (12.9 mg/ml) by oral inoculation, using the previously described procedure. Concentration of chicken IgG in the blood at specific time intervals was estimated by use of the chicken IgG-specific ELISA.

For phase 2, a total of 35 Large White pigs were used, and antibody feeding schedules were as fol-

[b] Maine Biological Laboratories, Waterville, Me.
[c] Shinetsu Chemical Co, Tokyo, Japan.
[d] BioProbe International Inc, Tustin, Calif.
[e] Cappel, Organon Teknika Corp, West Chester, Pa.
[f] Cryo M-Bed, Bright Instrument, Combs, England.
[g] Model OFT/AS-60, Bright Instrument, Combs, England.
[h] Somnopentyl, Pitman-Moore Inc, Mundelein, Ill.

Table 1—Enzyme-linked immunosorbent assay-determined titers of chicken IgG in the gastrointestinal tract and serum of pigs

Age after birth	Hour after IgG inoculation	Anti-K88 fimbrial antibody titer					
		Serum	Stomach	Duodenum	Jejunum	Ileum	Large intestine
10 h	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	12.5±2.5	14.3±7.8	14.7±5.9	19.8±8.2	4.5±3.5	0.8±0.6
	6	44.0±2.0	3.3±1.3	1.8±0.5	1.9±0.4	21.5±3.5	37.0±7.0
	24	56.0	1.0	1.0	1.0	2.0	40.0
3 d	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	< 0.1	28.5±1.5	17.5±2.5	19.3±0.7	16.0±3.0	1.3±1.3
	6	< 0.1	1.3±0.3	2.2±1.2	8.7±1.3	20.0	19.0±1.0
	24	< 0.1	0.4	< 0.1	0.9	1.3	32.0
6 d	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	< 0.1	14.7±0.3	32.5±7.5	18.5±1.5	0.5	< 0.1
	6	< 0.1	0.9±0.1	1.7±0.6	11.3±1.2	34.5±11.5	21.5±9.0
	24	< 0.1	< 0.1	< 0.1	1.0	1.9	28.0
21 d	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	< 0.1	14.8±0.2	22.3±15.2	33.0±13.0	0.8±0.6	< 0.1
	6	< 0.1	1.7±1.2	0.1±0.1	0.7±0.2	6.4±5.7	4.0±0.8
	24	< 0.1	< 0.1	< 0.1	< 0.2	< 0.1	3.0
28 d	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	< 0.1	3.6±0.7	2.6±1.3	4.6±0.9	0.4±0.4	< 0.1
	6	< 0.1	0.8±0.8	< 0.1	< 0.1	1.9±1.9	< 0.1
	24	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

lows: 6, 8, 10, 12, 18, 22, 26, and 34 hours after birth of pigs. Antibody preparation was given only once to a particular age group. Numbers of pigs per group were 1 at 6 hours; 8 at 8 hours; 3 at 10 hours; 7 at 12 hours; 7 at 18 hours; 7 at 22 hours; and 2 at 26 hours. Mean body weight of pigs ranged from 1.4 to 1.6 kg. Data obtained, using two 34-hour-old pigs, as well as four 10-month-old pigs in phase 1 of the experiment, were incorporated in this study. The concentration of egg yolk IgG used was 13 mg/ml, and the antibody preparation was inoculated orally in each pig by using the previously described procedure. Blood sample collection was done only on day 6 after administration of antibodies. Absorbed egg yolk IgG into the blood was assayed by use of chicken IgG-specific ELISA, and quantity of absorbed IgG was estimated.

Experiment 3: Passive protective effect of chicken egg yolk immunoglobulins against experimentally induced infection with ETEC—Fifteen Large White pigs were acquired at birth and were deprived of colostrum. Trial 1 consisted of 5 pigs administered 5 ml of chicken egg yolk antibody solution (anti-K88 fimbrial antibodies; titer 4,800) 10 hours after birth, then challenge-exposed with 10^{12} colony-forming units of K88$^+$ ETEC/pig 2 hours after pretreatment. Trial 2 consisted of 5 pigs administered 5 ml of chicken egg yolk antibody solution (anti-K88 fimbrial antibodies; titer 480) 10 hours after birth, and challenge-exposed with similar dose of K88$^+$ ETEC per pig 2 hours after pretreatment, and treated with antibody solution (titer 480) 3 times/d for 7 consecutive days. Trial 3 consisted of 5 control pigs administered phosphate-buffered saline solution only and challenge-exposed with similar dose of K88$^+$ ETEC per pig 12 hours after birth. The clinical response of each pig was recorded throughout the experiment and was analyzed in the context of these variables: fecal consistency score, weight loss, enumeration of infecting strains from rectal swab specimens, and mortality.[1] Experimental subject pigs subsisted on formula milk[i] given 3 times/d throughout the trial. The pigs used in this study was confined in clean isolation cages in a well-ventilated room with ad libitum access to water. All orally administered inocula, either antibody preparation or challenge bacteria, were administered by use of a syringe attached to a length of flexible silicone tube that was held in place in the mouth of pigs. The pigs were euthanatized by overdose of ultrashort-acting barbiturate.[h]

Statistical analysis—Pearson's correlation analysis was used to estimate and test the significance of the simple linear correlation coefficient R, which is a measure of the degree of linear association between serum IgG concentration and age of pigs. Dunnett's multiple-comparison test was used to determine significance of the differences in absorption of chicken egg yolk IgG among age groups of pigs.

Results

Experiment 1: Chicken egg yolk IgG in gastrointestinal tract of pigs (Table 1; Fig 1)—High concentration of egg yolk IgG (628 μg/ml) was detected in the stomach, duodenum, and jejunum by capture-binding assay when intestinal contents were assayed 2 hours after antibody administration in newborn and preweaning pigs. Six hours after administration, the antibodies had traveled distally to the ileum and proximal portion of the large intestine, and by 24 hours, remaining antibodies had reached the large intestine before being excreted in the feces. In pigs < 4 weeks old, the distribution pattern of ingested antibodies in the gastrointestinal tract at cer-

[1] SPF-LAC, Borden Inc, Hampshire, Ill.

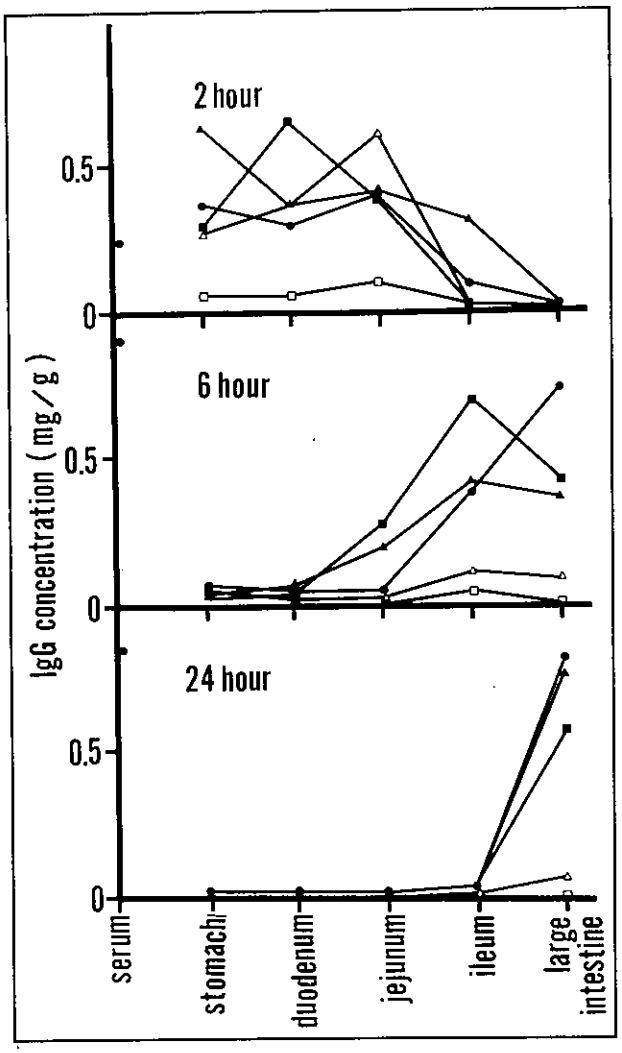

Figure 1—Egg yolk IgG concentrations in the gastrointestinal tract and chicken IgG concentrations in serum of pigs, as measured by ELISA. Antibody administration was done at 10 hours (●), and 3 (▲), 6 (■), 21 (△), and 28 (□) days after birth. Ingesta and blood samples were collected at 2, 6, and 24 hours after antibody administration.

tain time intervals did not vary as much, except in 28-day-old pigs, in which the passage of antibodies resulted in considerable loss of antibodies through digestion in the stomach. Subsequent assays of intestinal contents did not elicit a strong color reaction by ELISA. Chicken IgG concentration before administration was (100 mg/ml) and decreased several-fold after passing through the stomach (4 μg/ml) in 28-day-old pigs. Only traces of egg yolk IgG were detected throughout the intestinal tract of pigs in this age group after antibody administration. Results of IFA testing of tissue specimens correlated with ELISA results (Fig 2).

Experiment 2: Chicken IgG in the serum of pigs (Table 2; Fig 3)—Egg yolk IgG absorption varied considerably among pigs of different ages. Chicken IgG in serum of newborn pigs (10 hours old) was immediately identified 2 hours after oral administration of antibodies, and mean chicken IgG concentration increased within 24 hours. The peak concentration

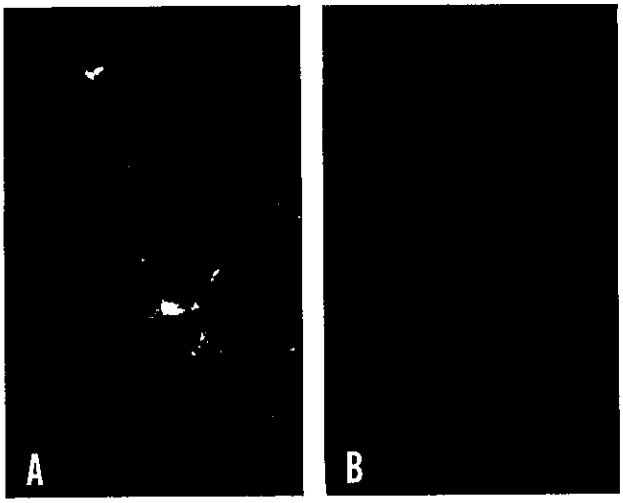

Figure 2—Micrograph of indirect fluorescent antibody testing of small intestine of a 10-hour-old pig, 2 hours after egg yolk IgG administration (A), and of the small intestine of a 12-hour-old control pig not fed egg yolk IgG (B).

in serum probably had been reached within 24 hours after antibody administration, and gradually decreased over a period of 21 days. Mean serum chicken IgG concentration in this group was 85.2 μg/ml, as measured by ELISA 2 hours after administration of antibodies. The egg yolk IgG was absorbed into the blood of young pigs as efficiently as colostral immunoglobulins until about 34 hours after birth. When antibodies were administered to 34-hour-old pigs, chicken IgG was not detectable in the serum 2 hours after egg yolk IgG administration, indicating that gastrointestinal tract closure could have occurred around that time. Older pigs (58- and 82-hours-old), likewise, had no detectable chicken IgG in serum after egg yolk antibody administration. Half-life data on serum chicken IgG concentration over time indicated an exponential decrease in concentration after the peak had been reached. If chicken IgG concentration in the serum is plotted on a logarithmic scale (Fig 3), a straight-line decrease is observed between 4 and 21 days. Absorbed egg yolk IgG has mean half-life of 1.85 days in pig serum, and chicken IgG concentration decreases to a tenth of its original concentration after 6.2 days and to a hundredth after 12.3 days in the blood. The process of gastrointestinal tract transfer is inhibited with increasing age of pigs; mean chicken IgG concentrations in serum of pigs that were fed egg yolk antibodies at 6, 8, 10, 12, 18, 22, 26, and 34 hours after birth were determined 6 days after antibody administration, and results indicate a straight-line exponential decrease in serum chicken IgG concentration over time (Fig 4). The rate of absorption of egg yolk IgG in the gastrointestinal tract decreased by half in 1.73 hours and, to a tenth in 5.75 hours, as calculated from the logarithmic serum IgG regression curve. Control pigs that were not administered egg yolk IgG had no detectable immunoglobulins in the serum.

Experiment 3—Protection was achieved in pigs (trial 2) that had daily antibody treatment after chal-

Table 2—Chicken IgG concentration (μg/ml) in the blood of pigs, as measured by ELISA

Time after birth (h)	Days after inoculation						
	0 (2h)	2	4	6	14	21	28
10	85.15±73.37	98.05±71.97	38.25±22.70	16.50±8.17	0.91±0.44	0.08±0.08	< 0.02
34	0.5±0.01	0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
58	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
82	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02

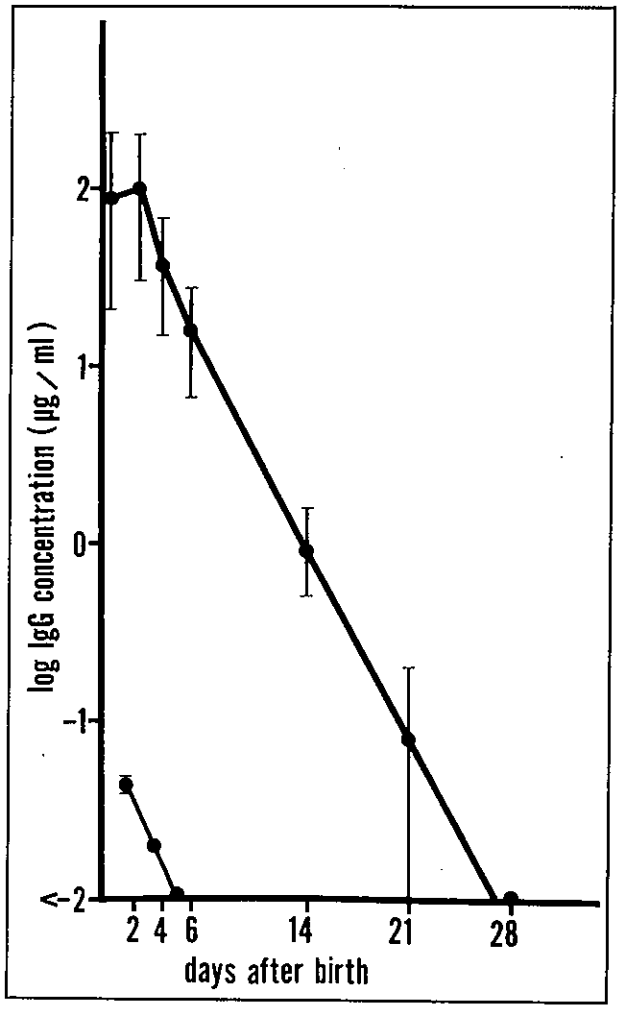

Figure 3—Measurement of chicken IgG concentration in the blood of pigs, as measured by ELISA. Egg yolk IgG half-life in serum was 1.85 days, $R = -0.9494$.

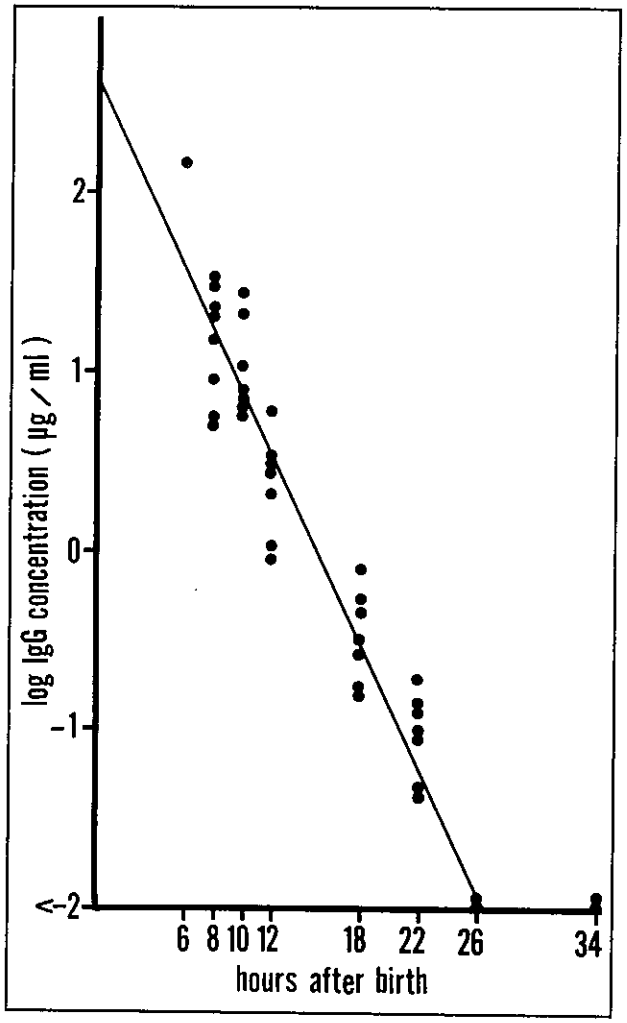

Figure 4—Measurement of chicken IgG concentration in the blood of pigs, as measured by ELISA. Egg yolk IgG absorption-inhibition time was 1.73 hours, $R = -0.9501$.

lenge exposure with K88+ ETEC strain; 100% survival was achieved. In contrast, pigs in trial 1 that were given a single dose of egg yolk IgG did not survive the experimentally induced infection, although high antibody titer was administered and high serum chicken IgG concentration was detected. Control pigs that were not given antibody died of *E coli*-induced enterotoxemia.

Discussion

Our data indicated that chicken egg yolk IgG can be absorbed and transferred as efficiently as colostral antibodies to the blood of neonatal pigs. Chicken IgG has a serum half-life of 1.85 days in newborn pigs. This is considerably shorter than the reported serum half-life of 12 to 14 days for homologous IgG (colostral antibodies).[13,14] Normally, the decrease in immunoglobulin concentrations in the blood is a function of protein catabolism and dilution attributable to increased body size and, hence, blood volume. Whether the faster disappearance of heterologous IgG from the serum of young pigs is caused by greater catabolism exerted on this foreign substance is not fully understood. The structural properties of chicken egg yolk IgG (eg, molecular size, conformation of IgG domains, intramolecular bonding, lack of disulfide linkage in the IgG L-chain, and lower flexibility of the hinge region) were considered to influence the overall properties of the immunoglobulin molecule and

are structural factors that might have bearing on the lower molecular stability of egg yolk IgG, compared with mammalian IgG.[15,16] Egg yolk IgG also is less rigid and stable under acidic conditions, and passage of the molecule in the stomach at low pH could weaken its overall conformational stability more readily than that of mammalian IgG, which could influence its faster rate of decay in the circulation.

The transport of immunoglobulin to the blood is known to cease within about 24 or 48 hours after birth.[17-20] Results of this study concurred with previous findings that cessation of immunoglobulin absorption from colostrum occurred at 34 hours of age, after a logarithmic decrease in absorption rate from birth. In this study, cessation of egg yolk IgG absorption and transfer to the circulation was observed around 34 hours of age. The fraction of egg yolk IgG absorbed into the circulation from the same dose of egg yolk IgG administered to pigs decreased with increasing age of pigs. Difference in the absorption rate of egg yolk IgG into the circulation between 8- and 22-hour-old pigs was significant ($P < 0.01$; Fig 4). Dilution of chicken IgG in the blood would have little influence on the results, because difference in blood volume between these age groups of pigs was not substantial.

Egg yolk IgG was detected in the intestine of neonatal pigs and, to a lesser degree, in weaning pigs. Because egg yolk IgG may be destroyed by high pepsin action in the stomach (pH 2.3) of older pigs, fewer antibodies can pass through the intestine without being digested or with their structure and function remaining intact. In young pigs, antibodies can readily pass the stomach without being damaged by the immature gastric environment, thus allowing absorption of structurally functional antibodies in the small intestine and transfer to the circulation system. Although serum immunoglobulin concentrations may be high and indicate a healthy systemic immune status of the animal, they probably do not reflect the local defense status of the intestinal immunoglobulin system, which is probably more important than serum immunoglobulin concentration in fighting enteric infection. This explains the mortality during our protection trial involving newborn pigs administered a single dose of high-antibody titer. Despite the high serum chicken IgG concentration detected, these pigs did not survive the infection. It can be assumed that most of the antibodies might have been transferred to the blood, with only a few antibodies remaining in the intestinal tract to fight the infection. To achieve protective antibody activity in the intestine, the supply of antibodies in the area must be sufficient to bind with the infective antigens. Good efficacy was achieved after daily treatment of susceptible pigs by oral administration of antibodies. Chicken egg yolk IgG is innocuous, and adverse effects were not observed in association with its use for treatment of infectious intestinal diseases in pigs. Because chicken egg yolk IgG is absorbed and transferred to the blood of young pigs as efficiently as homologous IgG, oral administration of egg yolk IgG, aside from reinforcing local intestinal immunity, also can enhance the overall immune status of young pigs, thereby conferring increased resistance against exposure to disease-causing agents in the environment.

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