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PRODUCTION OF NEONATAL HEMOLYTIC DISEASE IN NEWBORN PIGS BY ORAL ADMINISTRATION OF ANTI-PORCINE ERYTHROCYTE OVINE SERUM

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The minimum time of separating piglets from their mothers in order to prevent hemolytic disease in newborn pigs was investigated by examining the red blood cell count, packed blood cell volume, blood hemoglobin concentration and erythrocyte osmotic fragility of naturally suckled piglets which had been given anti-porcine erythrocyte ovine serum orally at different times after birth. The erythrocyte osmotic fragility test proved to be the most effective test to detect the hemolytic condition. Decrease in erythrocyte resistance was observed only in the piglets given the serum within 18 hours of life. From this result, it was recommended that piglets should be fostered by a non-sensitized sow other than their mother for 24 hours after birth to prevent the occurrence of neonatal hemolytic disease.

INTRODUCTION

It is well known that colostral immunoglobulins are absorbed from the intestinal mucosa during the early stage after birth[1]. The absorption of these large molecules ceases within a certain period due to a mechanism known as "gut closure". Blood group antibodies found in the colostrum are agents of hemolytic disease of neonatal piglets and are among the large molecules influenced by gut closure. Therefore, the onset of hemolytic disease is closely related to the absorption of antibodies in the maternal colostrum before gut closure occurs.

The purpose of this study was to estimate the minimum time necessary to foster piglets in order to prevent neonatal hemolytic disease. For this purpose we examined the clinical and hematological changes and erythrocyte osmotic fragility of naturally suckled piglets given anti-porcine erythrocyte ovine serum orally at different times after birth.

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MATERIALS AND METHODS

Anti-porcine erythrocyte ovine serum was prepared as the blood group antibody and then given intravenously or orally to newborn piglets at different times after birth. Judgement of the absorption of the antibody was made in accordance with the erythrocyte fragility test.

Preparation of blood group antibody

The blood was collected from apparent healthy swine aged 6 months old, washed several times with saline, and then prepared for 50% erythrocyte suspension with saline. This specimen was used as the antigen for obtaining the antibody. Namely 50 ml of erythrocyte suspension was inoculated intravenously a total of 5 times to 2 Corriedale ewes at 5 day's intervals. Seven days after the final inoculation, whole blood was collected and the serum was separated. The serum was kept at −20°C until use. The agglutinin titer of both ovine sera applied to the direct agglutination test with the swine erythrocytes was the same, ie., 1 : 256.

Animals

Forty-three piglets delivered from 6 sows were used; they were all born normally and had no clinical disorders. Differences of the values of the direct agglutination titer and the indirect antiglobulin test titer between the newborn erythrocytes and the serum of their dam were shown in table 1. Three sows had no agglutinin while 2 sows showed values of 1 : 1 in the direct agglutination test and 1 : 2–1 : 8 in the indirect antiglobulin test respectively. The piglets from the remaining one sow showed no clinical symptoms in spite of the ingestion of the colostrum; however, the agglutinin titer was not determined in this case.

TABLE 1 *Antibody titers of dams' sera to newborns' erythrocytes*

LITTER NO.	BREED	DA	IAT
1	L	NT	NT
2	HY	0	0
3	YBL	0	0
4	HL	0	0
5	YL	1:1	1:2–1:4
6	LH	0–1:1	1:4–1:8

Remarks: DA = Direct agglutination test, IAT = Indirect antiglobulin test, NT = Not tested, L = Landrace, HY = Hampshire × Yorkshire, YBL = Yorkshire × Barkshire × Landrace, HL = Hampshire × Landrace, YL = Yorkshire × Landrace, LH = Landrace × Hampshire

Administration of the antibody (ovine immune sera)

The plan of administration of the blood group antibody was shown in table 2. Piglets in litter 1 were divided into 2 groups. One group was intravenously given 20 ml of the ovine immune sera at 6 days old to confirm that the sera was able to cause hemolytic icterus, and the other group served as a control group.

All littermates in litters 2, 3, 4, 5 and 6 were divided into 2 or 4 groups, including one control group, in each litter. Each group consisted of 2 or 3 individuals. The control piglets in these litters were not administered the immune sera. The remaining piglets in these litters were given the sera at different times after birth, ie., 12 hours (litters 2 and 4), 15 hours (litters 4 and 5), 18 hours (litters 3, 4, 5 and 6), 24 hours (litters 3 and 5), 30 hours (litter 3), 36 hours (litter 2) and 60 hours (litter 2). The sera dose was 30 or 40 ml for the oral administration. Through out this experiment the piglets were allowed free suckling.

TABLE 2 *Animals and administration of the blood group antibodies*

LITTER NO.	NOS. OF ANIMALS		ANTIBODY ADMINISTRATION		
	Administrated	control	Course	Dose	Time
1	2	2	Intravenously	20 ml ;	6 days old
2	6	2	orally,	30 ml ;	12, 36, 60 hrs.
3	6	2	orally,	30 ml ;	18, 24, 30 hrs.
4	6	2	orally,	30 ml ;	12, 15, 18 hrs.
5	9	2	orally,	40 ml ;	15, 18, 24 hrs.
6	2	2	orally,	40 ml ;	18 hrs.

Items examined

Clinical symptoms and hematological changes and erythrocyte osmotic fragility were investigated in all the piglets ; the piglets inoculated with immune serum intravenously were examined several times during 18 days after the administration. The piglets administrated with the sera orally were investigated for 72 hours after the ingestion. Examination of the control animals was started simultaneously with that of the piglets which were given the sera first. The period of examination of the control animals ranged from 6 to 12 days of age in litter 1 and 12 to 84 hours of age in the other litter. The following methods of examination were used : THOMA-ZEISS for red blood cell (RBC) count, cyanmethemoglobin (CN-MetHb) method for blood hemoglobin (Hb) concentration, microhematocrit method for packed cell volume (PCV), CROSBY & FURTH method[4] for free Hb value in blood plasma and the method by GIFFIN & SANFORD[7] for erythrocyte osmotic fragility.

Results

Control animals

Hematological observations of the control animals were indicated in figure 1 (litters 2-6) and figure 2 (litter 1). The range of the RBC count and PCV and Hb concentration values were widely spread from 320-620 million/μl, 20-40% and 5-12 g/dl respectively (tab. 3). In the erythrocyte osmotic fragility test of the controls, the initial points of hemolysis ranged between 0.64 and 0.76% of NaCl concentration, and that of the complete hemolysis ranged between 0.38 and 0.52% (tab. 3). General features of these control animals were gradual decline in RBC count and PCV and Hb concentration, and normal resistance of the erythrocytes to hypotonic saline.

FIGURE 1 *Hematological observations of control piglets in litters 2-6*

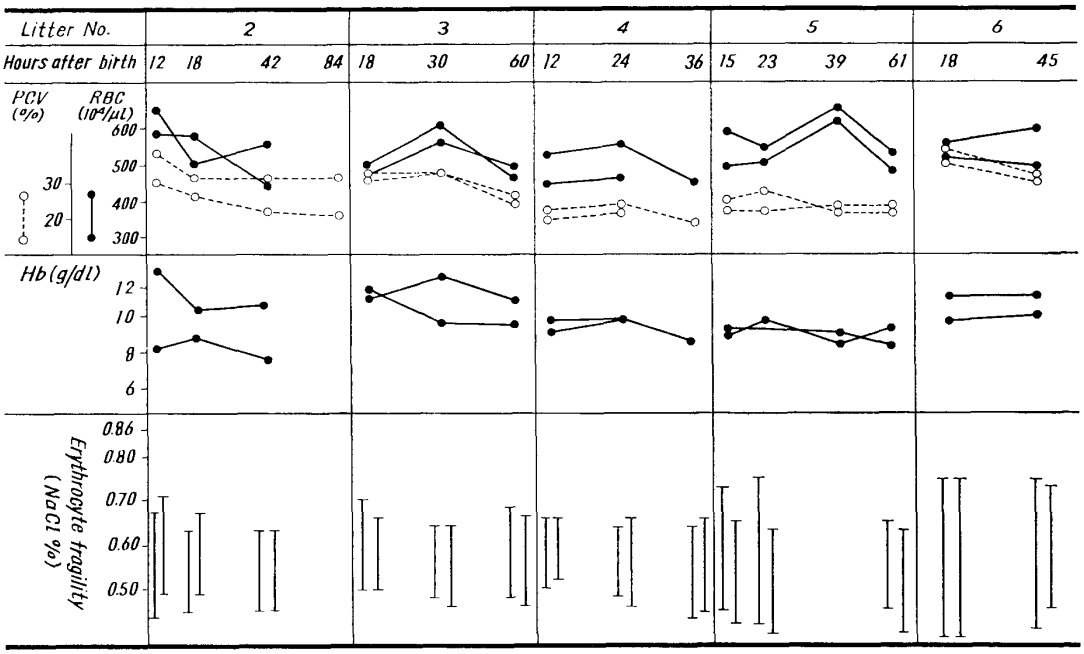


TABLE 3 *Ranges of estimated values of control piglets in litters 1-6*

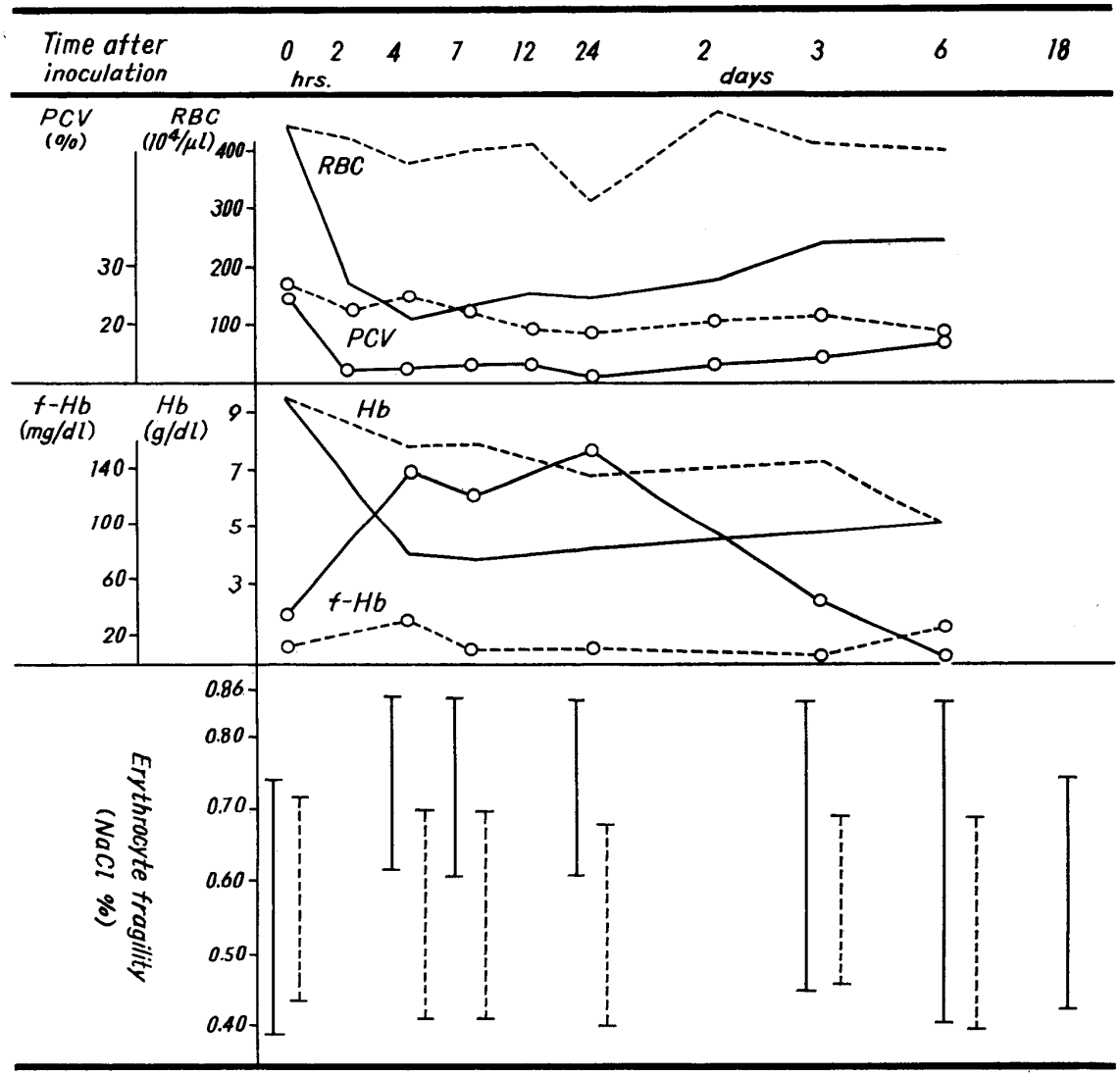
LITTER NO.	PERIOD EXAMINED	RBC COUNT ($10^4/\mu l$)	PCV (%)	Hb CONCENTRATION (g/dl)	ERYTHROCYTE FRAGILITY (NaCl%) Complete hemolysis	Initial hemolysis
1	6-12 days	320-450	20-27	5-10	0.40-0.46	0.68-0.72
2	12-84 hrs.	400-600	20-40	8-12	0.44-0.50	0.64-0.72
3	18-60 hrs.	475-600	23-32	10-12	0.46-0.50	0.66-0.70
4	12-36 hrs.	400-520	25-30	9-10	0.42-0.52	0.64-0.66
5	15-61 hrs.	450-620	27-35	9-10	0.40-0.44	0.64-0.70
6	18-45 hrs.	470-600	24-33	10-12	0.38-0.44	0.72-0.76

Intravenously inoculated animals

Hematological observations of the cases of intravenous inoculation (litter 1) were shown in figure 2. In this case, the piglets showed obvious anemia at 2 hours after the inoculation, and mild icterus was seen clinically at 4 hours. And these findings continued for 24 hours.

In the hematological observations, a drastic decrease in RBC count, Hb value and in PCV was seen in the inoculated animals which then recovered gradually. However,

FIGURE 2 *Hematological observations of litter 1 in intravenous inoculation of ovine immune sera*



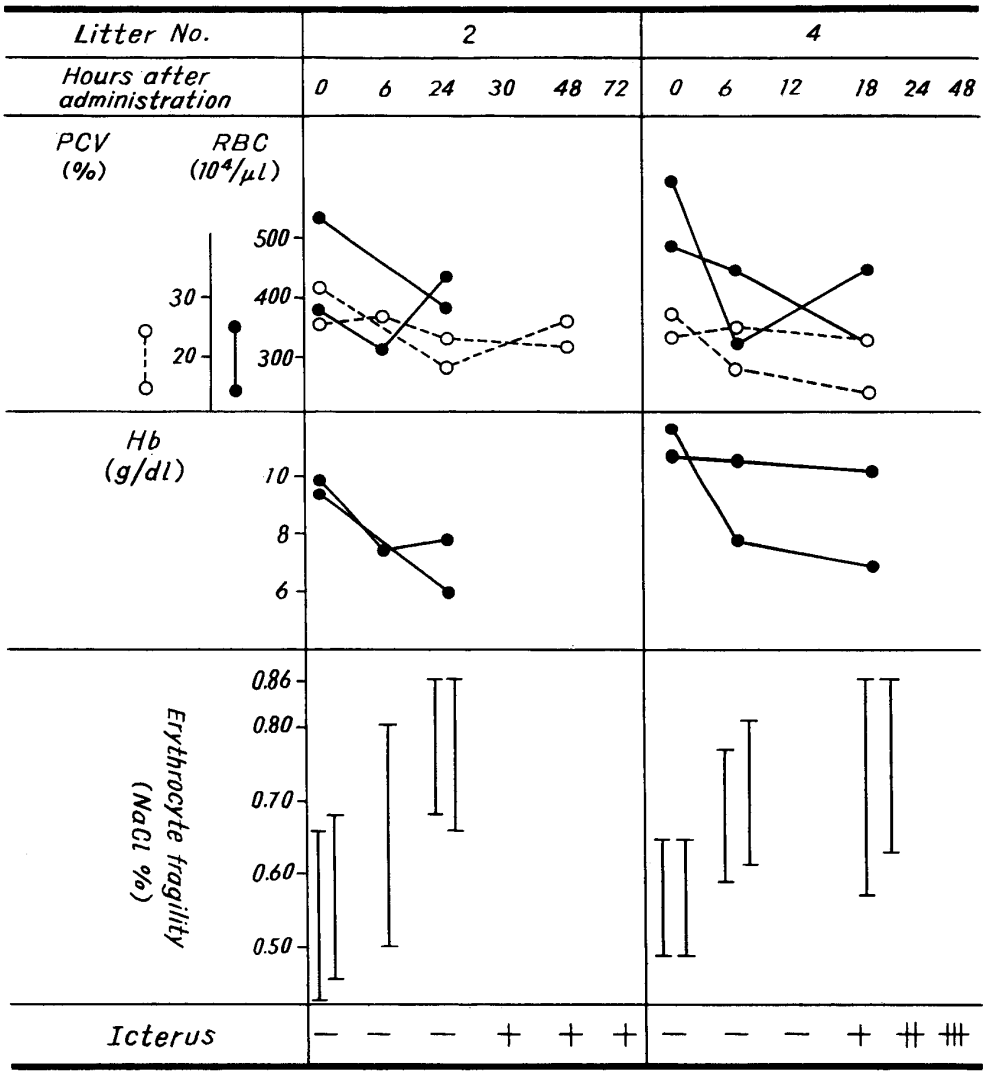
Remarks: Unbroken line=Inoculated piglets
Braked line=Control piglets
f-Hb=Free hemoglobin concentration in plasma

the RBC count was still lower at 6 days after the inoculation compared to the controls. On the other hand, free Hb concentration in the plasma was rapidly shifted up after the inoculation of the immune serum, reaching a peak during 4-24 hours. At 6 days later, it decreased to the normal value. The erythrocyte resistance became the weakest at 4 hours after the inoculation. The value for complete hemolysis returned to the same level of the control animals at 3 days after the injection, while the value for initial hemolysis was not recovered to the normal one until 18 days after the inoculation. No animal died during the experimental period.

Orally administrated animals

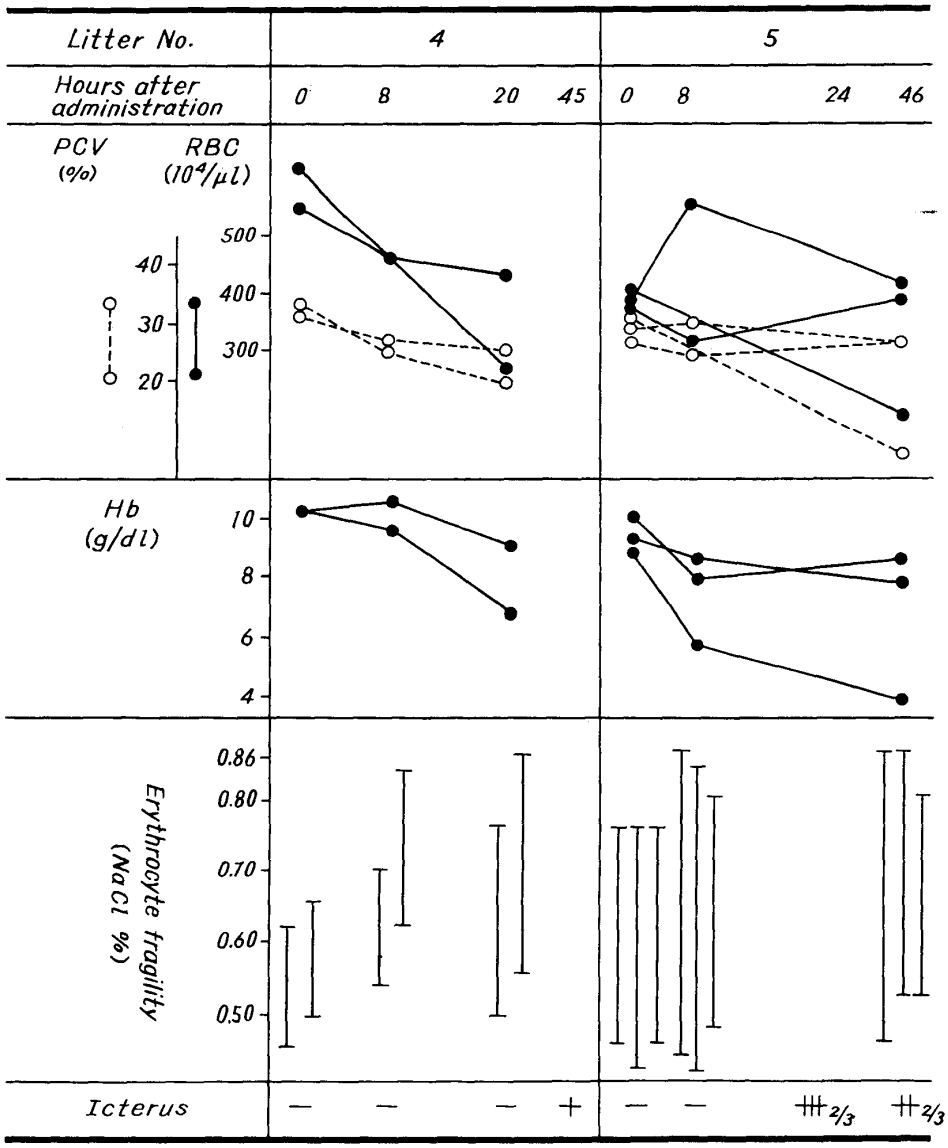
Hematological observations of the animals given the immune sera at 12 hours of

FIGURE 3 *Hematological observations of piglets given ovine immune sera at 12 hours of life*



life were shown in figure 3 (litters 2 and 4). In the administrated animals of this age, a trend of decrease in the RBC count and PCV and Hb concentration was also noticed; however, most of these values stayed within the ranges as were obtained from the estimation of the control animals (tab. 3). Only one exception, a piglet in litter 4, showed a low value of PCV below 20% at 18 hours after the administration. Characteristic features in these cases were a decrease in the resistance of the erythrocytes to hemolysis in hypotonic saline and the successive appearance of clinical icterus. Weakening of

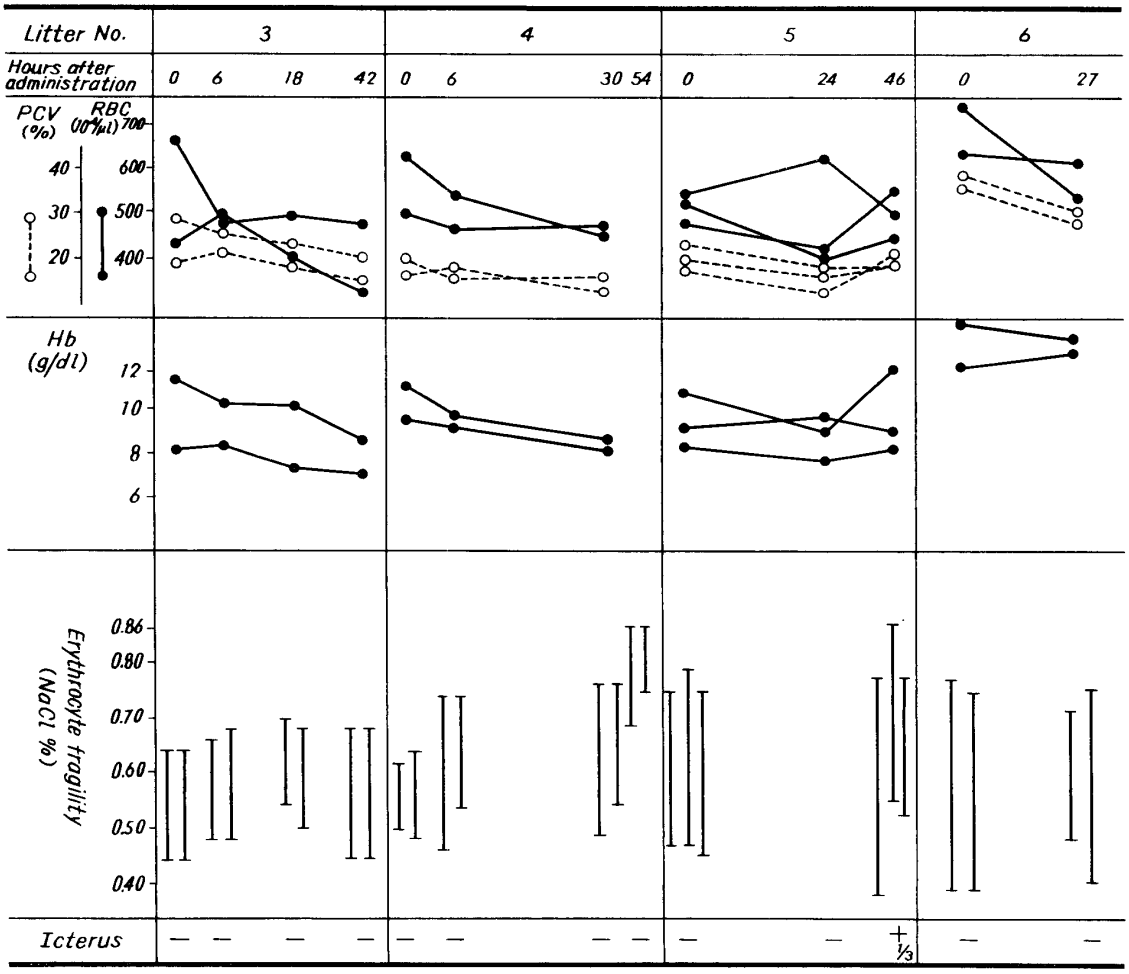
FIGURE 4 *Hematological observations of piglets given ovine immune sera at 15 hours of life*



the erythrocytes appeared within 24 hours after the ingestion of the sera and then reached a peak at which the initial hemolysis occurred at 0.86% of NaCl concentration in all animals. An increase in hemolysis of the erythrocytes in physiological saline was also noticed. The icterus appeared clinically at 30 hours (litter 2) and 18 hours (litter 4) after the administration and increased thereafter. Results of the erythrocyte fragility test and clinical findings showed that all piglets administered at 12 hours of age developed hemolytic condition.

The findings on the animals given the sera at 15 hours of age were shown in figure 4 (litters 4 and 5). One of the 2 animals in litter 4 showed lower values in the RBC count and PCV than those of the control animals (tab. 3) at 20 hours after the administration, and its erythrocytes began to hemolyze at 0.86% of NaCl concentration. At 45 hours after the administration, all animals of this litter showed clinical icterus

FIGURE 5 *Hematological observations of piglets given ovine immune sera of 18 hours of life*



and an increase of the hemo ytic color of erythrocytes in the physiological saline. In litter 5, one of the 3 animals showed a marked decrease in RBC count, PCV and Hb concentration. The point of initial hemolysis after the administration ranged between 0.80 and 0.86%. Increase of the hemolytic color and clinical anemia with icterus were observed in 2 animals at 46 hours after the ingestion. A decrease with time in erythrocyte resistance was a common finding in all piglets of these groups in litters 4 and 5.

Results from the piglets administered the sera at 18 hours of life were shown in figure 5 (litters 3, 4 and 5). The values of RBC count, PCV and Hb concentration were kept within the estimated ranges of the control animals except for the PCV of

FIGURE 6 *Hematological observations of piglets given ovine immune sera at 24 hours of life*

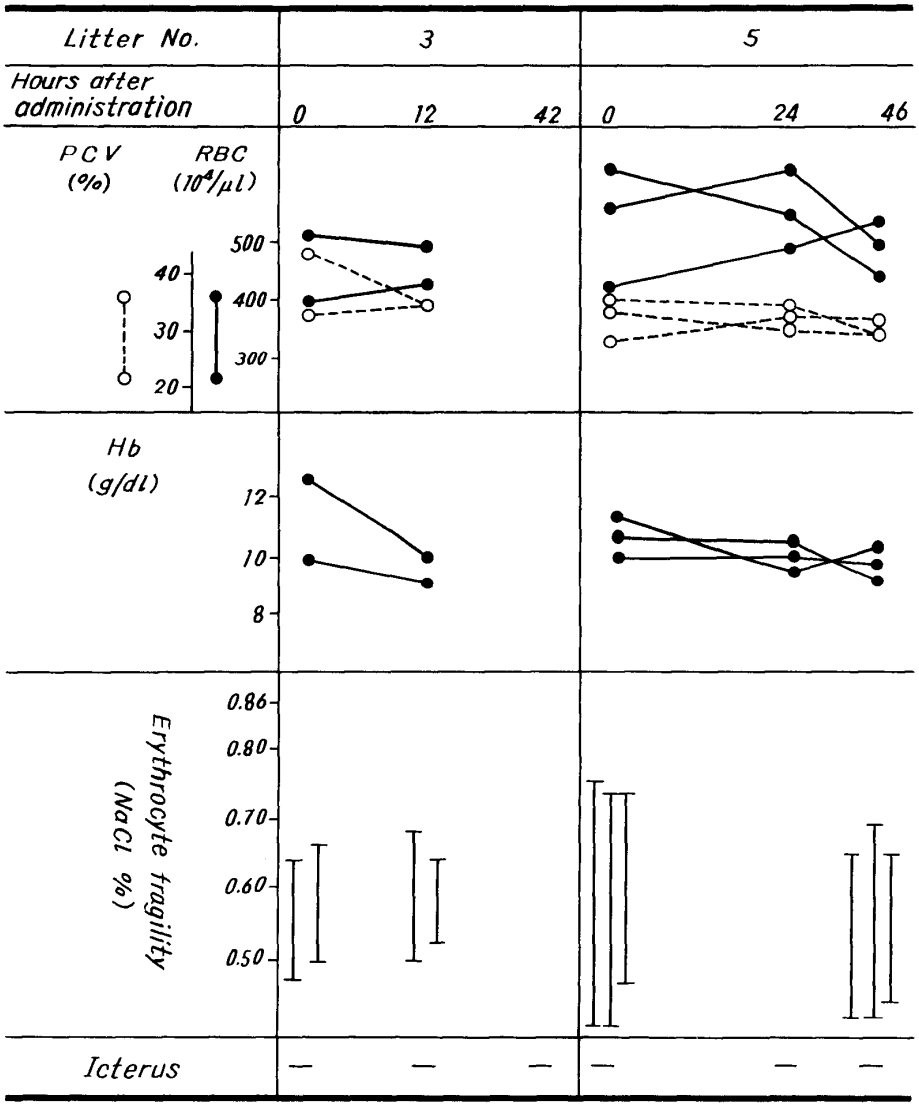
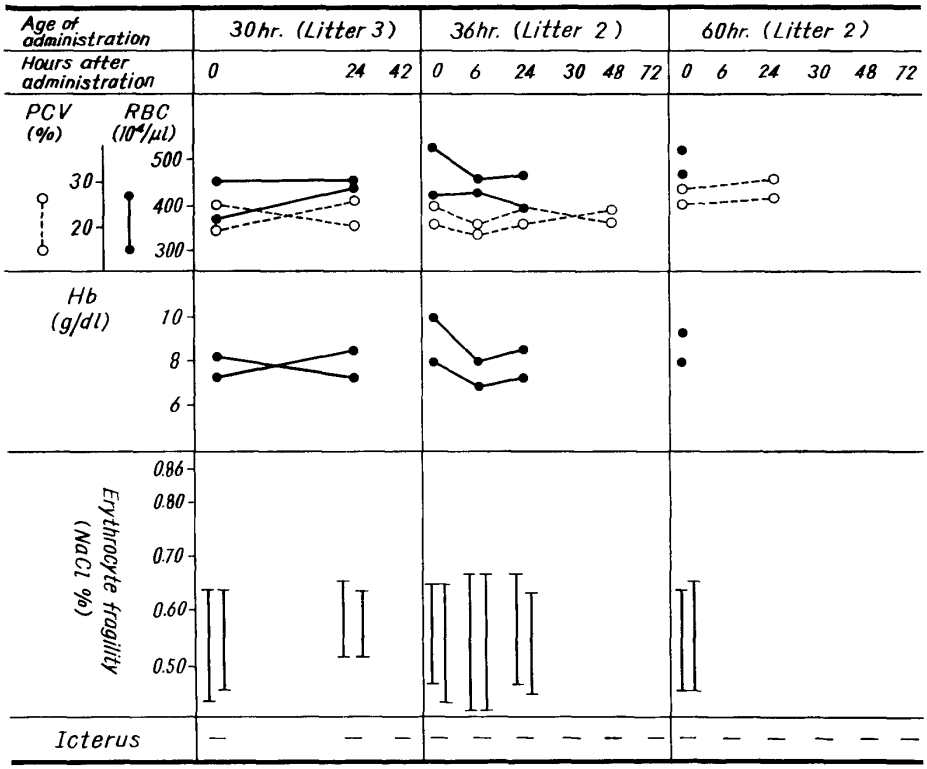


FIGURE 7 *Hematological observations of piglets given ovine immune sera at 30, 36 and 60 hours of life*



2 animals in litter 4 at 30 hours, while three animals from litters 4 and 5 showed hemolysis in 0.86% NaCl solution at 54 hours (in litter 4) and 46 hours (litter 5). Clinical icterus occurred at 46 hours in only one piglet of the litter 5.

The piglets which were administered the sera after 24 hours of life showed no abnormal hematological changes (figs. 6 and 7) and no clinical signs.

DISCUSSION

In 1949 BRUNER et al.[2] experimentally produced hemolytic anemia in newborn pigs by immunizing pregnant sows against the erythrocytes of the boars which bred them. Their experimental findings indicated that this disease occurred through the mechanism of antigen-antibody reaction between the erythrocytes of newborn pigs and the colostral antibody of sensitized sows. They also reported that one group of piglets did not develop anemia when they were suckled by their nonimmunized true mother for 2 days after birth and then fostered by another immunized sow, even though the sow's serum strongly agglutinated their erythrocytes in vitro. This fact suggested that the occurrence of this disease could be detected within an early limited stage and that it could be prevented by keeping newborns from drinking the colostrum containing the antibody for 2 days after birth.

On the other hand, many studies were carried out concerning the passive immunity of pigs[1,18,23]. Some wokers established that the capacity to absorb antibodies from the gut contents is lost at an early stage after birth due to a phenomenon called "gut closure", which correlates with morphological changes such as formation of the terminal web in the apical cytoplasm and disappearance of pinocytosis in the intestinal epithelial cells[12,13,15,21,22]. There are many reports suggesting that cessation of the immunoglobulin uptake by the small intestine occurs from 8 to 36 hours after birth in naturally suckled newborn piglets[8,14,17,19,20,24]. However, retardation of gut closure by starvation or feeding of small molecular substances such as glucose and amino acid mixtures has been achieved by some scientists[10,16,17]. In fact, PAYNE & MARSH (1962)[17] reported a piglet which showed absorption of antibody even at 106 hours of life when the animal was not allowed to suck its dam's milk.

As described above, it is thought that protection against this disease may be achieved by separating piglets from their mothers and not feeding them their mother's milk until the gut closure is completed.

Since the time in which gut closure operates is influenced by the size, the form of intaked matter and the time of ingestion are important factors. In this regard, several studies were carried out to ascertain the minimum time during which piglets of iso-immunized sows should be deprived of maternal milk in order to survive in various conditions. BAXTON et al. (1955)[3] returned piglets to their mother at 12, 18 and 24 hours of age after artificial feeding and found that all animals showed a positive reaction to the anti-globulin sensitization test and clinical signs of various degrees at 6 or 12 hours after drinking their mother's milk. They concluded that different pigs seemed to display variations both in their capacity to absorb iso-antibody from the digestive tract and in the period over which the absorption took place. HIMENO et al. (1968)[10] also removed piglets from their sow and gave them water only, artificial milk or cows milk 6, 8, 9, and 10 times at the rate of once every hour and then returned them to their mother. They found that the group fed 6 times developed anemic and icteric conditions while the piglets fed 8, 9 and 10 times survived vigorously without any clinical signs. They thus recommended the 10 times administration of artificial milk for control of this disease to allow for the individual difference. As shown, the results of the above experiments differed from each other; the differences can probably be attributed to variations in the feeding conditions and the methods employed for detection of the onset of the disease.

The method used in our experiment differed from BRUNER's[2] in the point of the use of immunized ovine serum instead of immunized sows milk for easy obtaining of high titer antibody against porcine erythrocyte. There has been no report on the production of the hemolytic disease by direct administration of these heterologous anti-serum to newborn piglets. In one case of experiments with rabbits[5,6], clinical symptoms

of various degrees were observed following the administration of the serum ie., 1 ml/kg body weight caused death with shock syndrome. In our experiment, no shock syndrome was observed, but clear hemolytic anemia appeared when 20 ml of the immunized serum was inoculated intravenously and orally to the piglets. In spite of the smaller amount of the serum compared with the natural dose of the colostral immunoglobulin,[9] clear hemolytic anemia was seen when intravenous inoculations of 20 ml of the immunized serum were used. This fact revealed that the antiserum, which was employed in oral administration, possessed an ability to cause clear hemolysis when it flowed into the blood stream.

In the present experiment, RBC count, PCV and Hb concentration and erythrocyte fragility were examined in addition to clinical changes to detect the onset of hemolytic anemic condition. Among these items, RBC count and PCV and Hb concentration in the control animals showed a trend of gradual decline with time. This drop might have resulted from blood dilution after the intake of colostrum. Thus this trend might account for the abnormal values of these items in hemolytic condition. In contrast with this trend, erythrocyte resistance remained constant in the control animals. And as compared to results of our previous studies[11], we found some cases showing a weakened erythrocyte resistance without clinical signs. Therefore, the result of the erythrocyte osmotic fragility test was considered to be useful to detect the hemolytic condition. Judging from the result of the erythrocyte osmotic fragility test, decrease of the erythrocyte resistance occurred only in the animals given the ovine serum within 18 hours of life. Since the administration of the serum at 18 hours of life induced a decrease of the erythrocyte resistance in three animals of the nine piglets, this age was considered to be a turning-point, from a sensitive to an insensitive phase, under this experimental condition. In other words, this result suggests that removal of piglets from the immunized mother for 24 hours is necessary to prevent the onset of the disease when neonatal piglets are fostered by non-sensitized sows.

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