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CLINICO-HEMATOLOGICAL STUDIES ON SUBCLINICAL CASES OF NEONATAL HEMOLYTIC DISEASE IN PIGS

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Clinico-hematological aspects were studied in 5 litters in which no anemia and icterus were observed clinically to characterize the subclinical changes of neonatal hemolytic disease in pigs. The findings are summarized below.

1) Subclinical cases with various degrees of the disease were revealed.

2) Decrease of the resistance of erythrocytes to hemolysis was observed in all animals soon after the initial suckling. The red blood cells were hemolytic even in physiological saline.

3) Relatively mild decline was noticed in the red blood cell count and the packed blood cell volume and blood hemoglobin concentration along with an increase of free hemoglobin concentration in the plasma.

4) It was concluded that subclinical cases may appear in relation to the amount of antibody ingested from the colostrum: (1) if the milk contains a low level of antibody in spite of a large intake of colostrum; or (2) if in a small intake of colostrum there is a high titer of the antibody present.

INTRODUCTION

In 1949, BRUNER et al.[5] demonstrated experimentally the occurrence of hemolytic disease in neonatal piglets, and in the next year, KERSHAW (1950)[21] reported 10 field cases of the disease in England. Following these, many reports concerning other outbreaks of the disease[6,7,10,29,31] and the relationship between hemolytic disease and the blood group of sows, boars and their offspring were added to the literature[4,8,12,24,26]. In Japan, hemolytic disease has been studied in field cases from the perspective of immunological and pathological backgrounds[1,3,14~19,27,28]. On the other hand, few studies have been carried out from the clinico-hematological point of view already expressed.

The clinical symptoms of piglets infected with hemolytic disease are variable and clinical findings and prognosis are not always typical; some littermates, for example,

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may survive without showing symptoms while others suffer acute death with severe hemolysis[1]. It is difficult to determine from the clinical findings whether these littermate possesses stem from the same genetical factors or not.

The authors carried clinico-hematological studies on piglets without symptoms to clarify the criteria of subclinical cases of hemolytic disease in neonatal piglets.

MATERIALS AND METHODS

Animals

The animals employed in these studies consisted of 5 litters and their sows. Details of the animals are given in table 1.

TABLE 1 *Antibody titers in subclinical cases*

LITTER NO.	BREED	PARTURITION	LITTER SIZE	ANTIBODY TITER AGAINST PIGLETS' ERYTHROCYTES					
				Dam Serum		Dam Colostrum		Boar Serum	
				DA	IAT	DA	IAT	DA	IAT
1	H	3rd	12	32	64	NT	NT	128	256
2	H	7th	8	16	64	128	512	32	128
3	HY	3rd	7	2	4	4	16	NT	NT
4	HY	2nd	7	4	8	32	0	NT	NT
5	HY	3rd	10	1	0	4	4	NT	NT

Remarks: H=Hampshire, HY=Hampshire×Yorkshire, DA=Direct agglutination test, IAT=Indirect antiglobulin test, NT=Not tested

Hematological examination

The blood of sows was taken from the auricular vein or the *vena cava*. Red blood cell count was determined using THOMA-ZEISS method. Hemoglobin (Hb) concentration was estimated using the cyanmethemoglobin (CN-MetHb) method. Packed blood cell volume (PCV) was determined according to the micro-hematocrit method. Icterus index was examined on the plasma with MEULENGRACHT's method. Total serum protein level was determined using a refractometer (Hitachi, Co., Ltd.). The level of the serum γ-globulin was estimated with electrophoretic method using cellulose acetate membrane. The erythrocyte osmotic fragility test was carried out by the method of GIFFIN & SANFORD (1918)[11] as follows. From the heparinized blood samples, 10% suspension of red blood cell was prepared with physiological saline. Then, one drop of this suspension was dropped into each tube containing NaCl solution ranging from 0.36 to 0.86% with 0.02% interval. The tubes were kept for 2 hours at room temperature, and then the points of the initial and complete hemolysis were read. The color score of hemolysis was determined using the electrophotometer with 540 nm wave,

after adding 0.02 ml of blood to 3 ml of physiological saline and keeping it for 1 hour, the diluted blood was centrifuged at 3,000 r. p. m. for 5 minutes. Free Hb concentration of the plasma was examined by the method of CROSBY & FURTH (1956)[9].

Serum reaction

The direct agglutination test and the indirect antiglobulin test were carried out on the maternal sera and the whey of the colostrum and on the erythrocytes of the littermates derived from the sows tested and the boar which sired them.

For the production of anti-globulin sera, whole porcine serum was used as the antigen, and 2 ml of the antigen was inoculated 7 times intravenously into a rabbit at 3-4 days intervals. Whole rabbit blood was collected 7 days after the final inoculation and then the immune serum was separated from the blood. The collected sera were inactivated at 56°C for 30 minutes, and to remove the hetero agglutinine against the swine erythrocytes, the antisera were absorbed with pooled porcine erythrocytes which had been washed 7-8 times with physiological saline. The whey of the colostrum was prepared with adding the calf rennet after LECCE & LEGATSE (1959)[22].

The procedure of the direct agglutination test was as follows: (1) mix one drop of 2% suspension of the piglets' red blood cell to 2 drops of the serially diluted maternal serum or to 2 drops of the similarly treated whey; (2) centrifuge these mixture at 1,000 r. p. m. for one minute after 30 minutes; (3) determine the agglutination titer. After the reading of the agglutination reaction, the same test tubes were allowed to stand at 37°C for 30 minutes and then washed 3 times with physiological saline to obtain the 2% erythrocyte suspension again. Two drops of the anti-globulin serum, which was diluted thirtyfold with physiological saline, were added to the red blood cell suspension. And then the reaction was observed after remaining at room temperature for 10 minutes and centrifugation at 1,000 r. p. m. for 1 minute.

Intake volume of colostrum

The littermates were reared separately from their sow and allowed to suck sows milk at intervals of 1 hour. The piglets' weight was examined before and after the suckling, and the differences were estimated as intake volume of the colostrum.

RESULTS

Litter 1

The 12 piglets in this litter were born normally. Four of them were allowed to suckle another sows milk as foster animals. All littermates were small in size and relatively weak. No icterus color was seen in any visible mucosa. Intake volume of colostrum was small in every piglet measured; these data were given in tables 2 and 3. Total volume of colostrum intake examined from birth to 8 hours old was very low in all the piglets. As to the relationship between colostrum intake, total serum protein

and γ-globulin, a higher level of serum total protein and γ-globulin were seen in piglets Nos. 1, 2 and 6, which intook more colostrum as compared to the other littermates. On the other hand, the piglets which intook much colostrum and showed a high level of total serum protein were more than 1 kg in body weight while the other piglets were less than 1 kg.

The red blood cell count of the piglets with high total serum protein was relatively low during the first 12–24 hours after birth, and conversely, the icterus index was high during the same period. This fact suggests that the antibody was already present at this time. Of the 3 piglets with hemolytic icterus, 2 survived and 1 died at 16 days old.

TABLE 2 *Body weight at birth, intaken colostrum and serum protein of litter 1*

PIGLETS NO.	BODY WEIGHT (kg)	INTAKEN COLOSTRUM*1 (g)	SERUM TOTAL PROTEIN (g/dl)		γ-GLOBULIN (g/dl)		PROGNOSIS
			12 hrs.	24 hrs.	12 hrs.	24 hrs.	
1	1.15	83	5.7	6.9	2.3	2.7	died
2	1.05	89	6.4	•	3.1	•	survived
6	1.25	99	7.0	6.5	2.9	2.7	survived
7	0.40	29	3.1	•	0	•	died
9	0.70	45	3.2	2.7	0	0	died
10	0.75	40	4.3	5.1	1.0	1.3	died
11	0.55	19	4.3	3.7	0.9	0.8	died
12	0.65	14	3.0	6.0	0.3	2.8	died

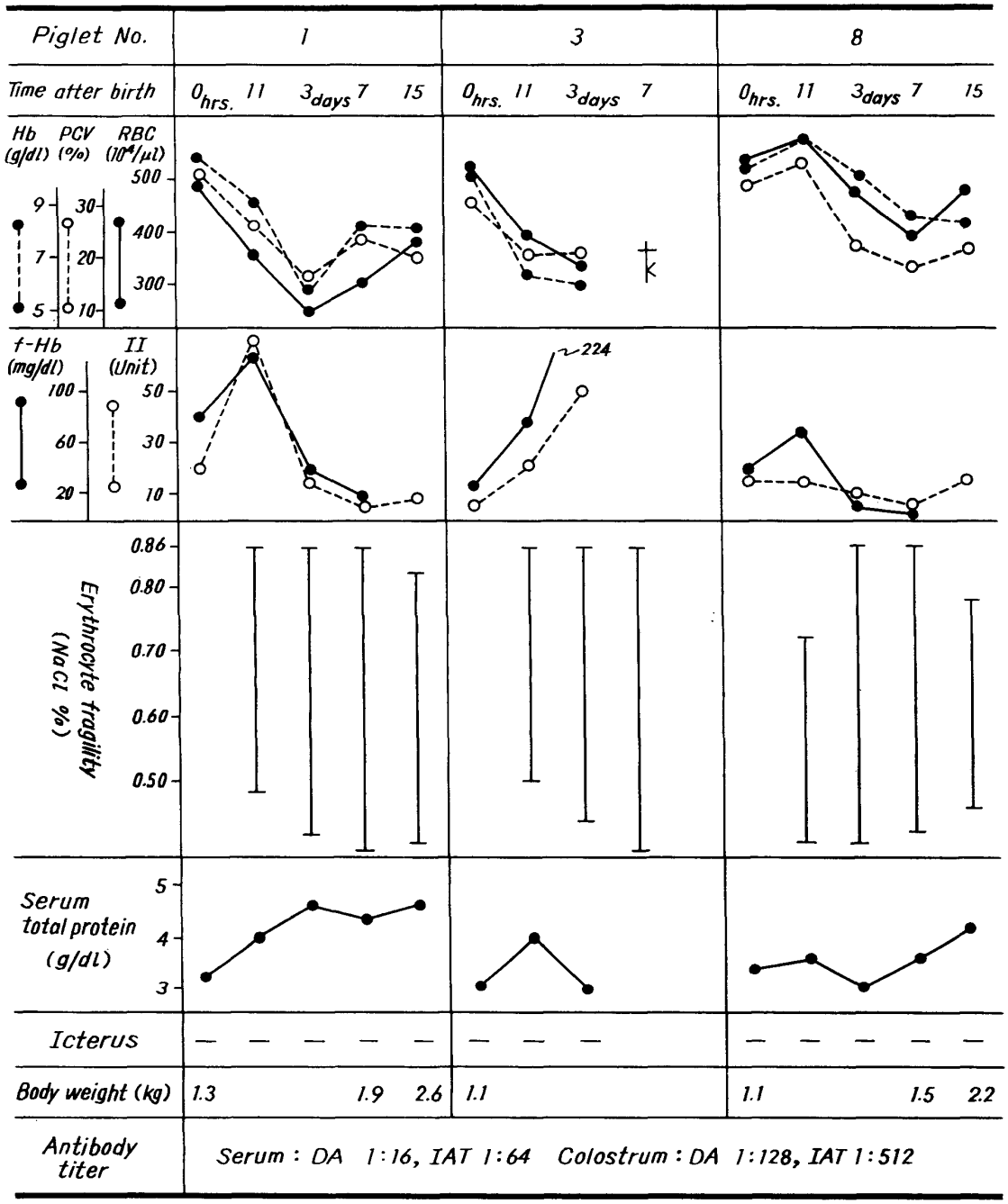
*1 Within 8 hours of life

TABLE 3 *Intaken colostrum, red blood cell count and icterus index of litter 1*

PIGLET NO.	INTAKEN COLOSTRUM*1 (g)	RBC ($10^4/\mu l$)		ICTERUS INDEX	
		12 hrs.	24 hrs.	12 hrs.	24 hrs.
1	83	248	294	7.0	9.0
2	89	421	281	15.0	•
6	99	533	329	20.0	15.0
7	29	654	555	3.0	3.0
9	45	321	610	3.0	3.0
10	40	583	639	7.0	6.0
11	19	463	463	3.0	3.0
12	14	430	467	6.0	6.0

*1 Within 8 hours of life

FIGURE 1 *Hematological observations of litter 2*



Remarks: f-Hb=Free hemoglobin concentration in plasma
II=Icterus index
DA=Direct agglutination test
IAT=Indirect antiglobulin test

On the other hand, the other 5 piglets which intook only a small amount of colostrum died within 2 to 23 days of age. The erythrocyte osmotic fragility test was not done for these littermates.

Litter 2

Though the 8 piglets in this litter were born normally, three died immediately after birth before colostrum intake. Clinical and hematological examinations were carried out for the remaining 5 piglets from birth to 15 days of age. On the whole, the piglets examined were weak and low in weight. In all piglets, no icterus and anemia were observed in the visible mucosa during the experimental period.

In figure 1, the hematological characteristics and antibody titer of the colostrum whey and maternal sera against the erythrocytes of their piglets were shown. The antibody titer of maternal sera with the direct agglutination test was 1 : 16 and that of the colostrum was 1 : 128, while in the former antibody titer measured with the indirect antiglobulin test was 1 : 64 and that of the latter was 1 : 512 respectively. These results indicated that the dam possessed a relatively high titer of incomplete antibody.

In the hematological examination, piglets Nos. 1 and 3 showed a low red blood cell count and a low level of PCV and Hb after their intake of colostrum, and these values were lowest at 3 days of age. On the other hand, the free Hb concentration and icterus index of the sera were raised at 11 hours after colostrum intake, and especially in piglet No. 8, the red blood cell count, PCV and Hb concentration were relatively high at 11 hours after colostrum intake as compared to those of piglets Nos. 1 and 3. However, these levels of piglet No. 8 gradually became lower, reaching the lowest value at 7 days old. In this piglet, there was only a slight increase in the plasma free Hb level and no rise in the icterus index was seen.

In the erythrocyte fragility to hypotonic-saline, piglets Nos. 1 and 3 showed increases at 11 hours after colostrum intake while animal No. 8 showed the lowest resistance to hypotonic-saline at 3 days old.

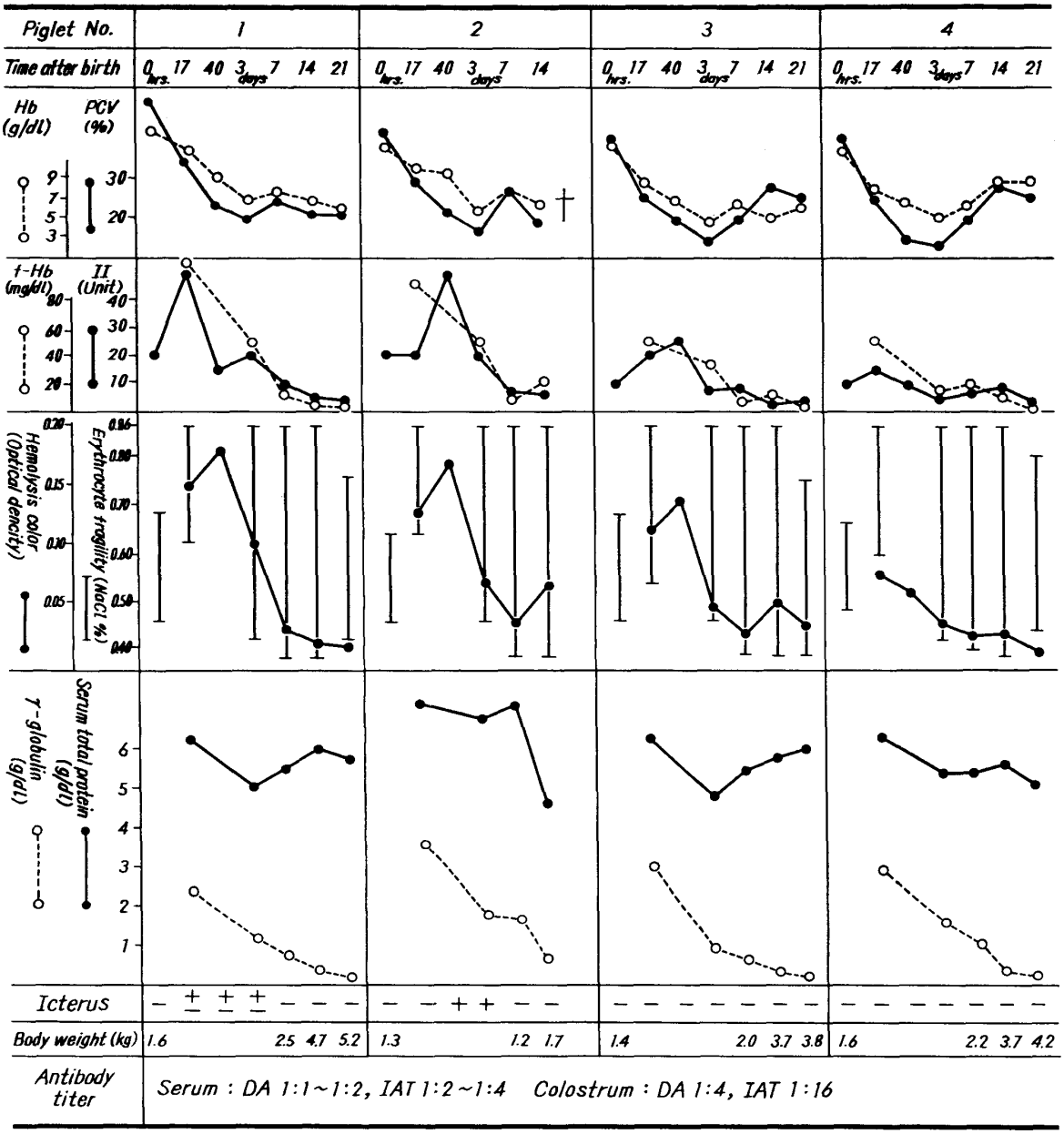
Total protein level of piglets Nos. 1, 3 and 8 was less than 4 g/dl at 11 hours old; this result indicates that only a small amount of colostrum was taken by these piglets.

As was mentioned above, in these 3 piglets no hemolytic icterus was seen clinically, although increase of the erythrocyte osmotic fragility and of plasma free Hb concentration was obvious. This finding indicates that only slight hemolysis was present in these cases.

Litter 3

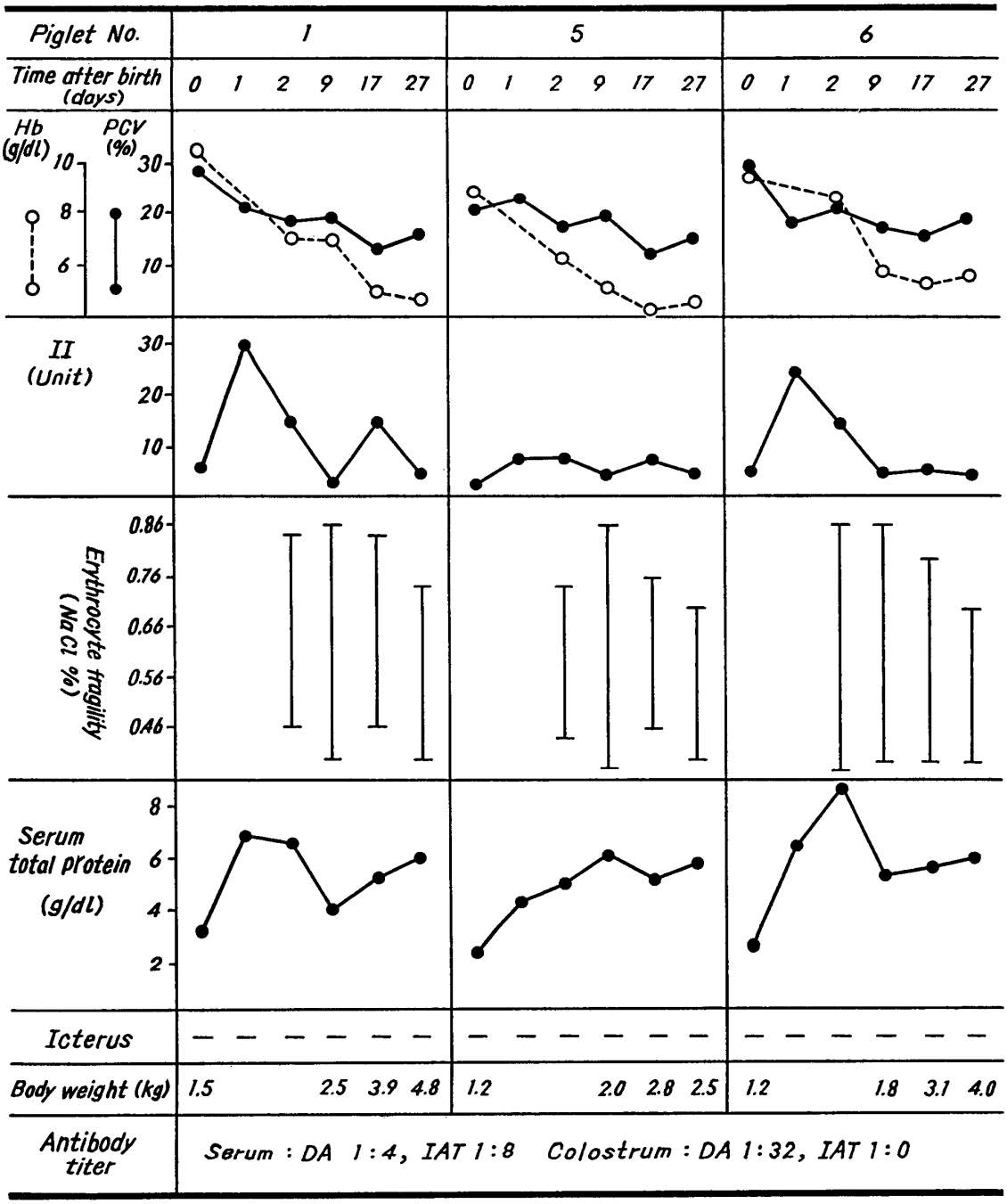
Of the 7 piglets which were normally born, 4 were used for clinical and hematological observations; the results were summarized in figure 2. There were no clinical symptoms in these piglets with the exception of piglets Nos. 1 and 2 which showed

FIGURE 2 *Hematological observations of litter 3*



Remarks: f-Hb=Free hemoglobin concentration in plasma
II=Icterus index
DA=Direct agglutination test
IAT=indirect antiglobulin test

FIGURE 3 *Hematological observations of litter 4*



Remarks: II=Icterus index
DA=Direct agglutination test
IAT=Indirect antiglobulin test

very mild icterus in the conjunctiva. The levels of antibody in the maternal serum and the colostral whey against the piglets' erythrocytes were relatively low, ie., 1 : 16.

Hematological observations revealed that the PCV and Hb concentration decreased after colostrum was ingested, and these values were lowest at 3 days of age. In piglets Nos. 1 and 2, which showed mild icterus after birth, the maximum rising of plasma free Hb concentration and the icterus index were observed at 17–40 hours after the initial suckling, and then these levels decreased gradually. In piglets Nos. 3 and 4, there were no visible icterus symptoms and only slight rising of the icterus index. On the other hand, maximum and minimum resistance in the erythrocyte osmotic fragility test decreased to an extraordinary degree in all the piglets. Serum total protein and γ-globulin level in each piglet showed values of more than 6 g/dl and 2 g/dl respectively ; these results revealed that all piglets had ingested colostrum promptly after birth.

Litter 4

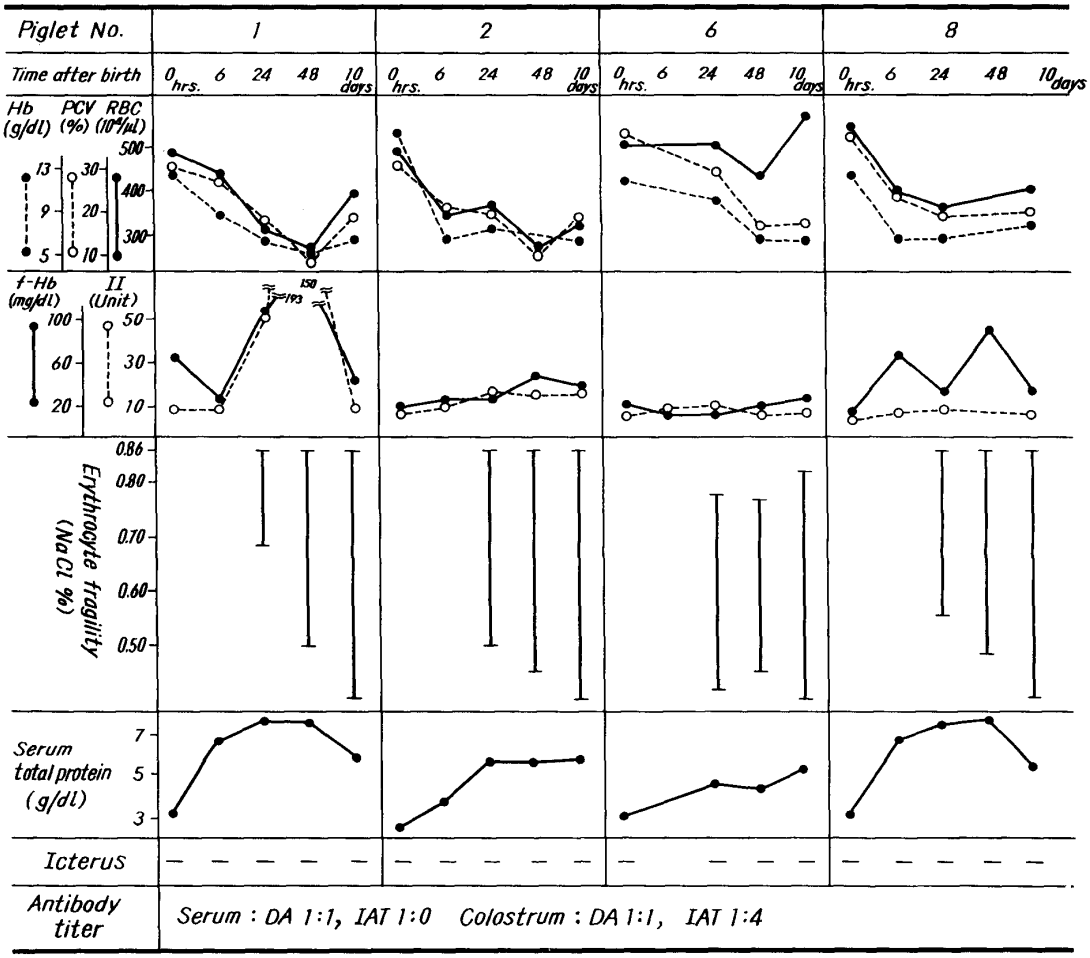
All littermates, 7 piglets in total, were born normally, and there were no obvious clinical symptoms throughout the experiment. Antibody titers of maternal serum and the whey of colostrum were 1 : 4–1 : 32 in the direct agglutination test and 1 : 0–1 : 8 in the indirect antiglobulin test.

Three of the 7 piglets were used for successive hematological observations ; the results were shown in figure 3. After the initial suckling, decreasing of PCV and Hb concentration were observed along with a more descending Hb concentration as compared to that of PCV. There was no obvious icterus on the visible mucosa in any of the piglets. The icterus index of piglets Nos. 1 and 6 rose 1 day after the initial intake of colostrum. There was no rising of the PCV and Hb concentration observed in No. 5. On the other hand, there was a clear decrease of minimum resistance in the erythrocyte osmotic fragility test noticed in piglets Nos. 1 and 6 at 2 days after the initial suckling, whereas no such decrease was observed in No. 5 until 9 days after birth. There was no decrease of the maximum resistance to the erythrocyte osmotic fragility test found in any of the piglets examined throughout the experimental period. The levels of total serum protein in Nos. 1 and 6 was more than 6 g/dl at 24 hours after birth, which revealed that these piglets were given enough colostrum, whereas the concentration was 4.3 g/dl in piglet No. 5, indicating that this piglet received less colostrum than the others.

Litter 5

All 10 piglets in this litter were apparently born in good health ; 4 piglets of this grower were observed hematologically, and the results were shown in figure 4. In these piglets, the PCV and Hb concentration were gradually decreased after 48 hours of age, and the levels of total serum protein were highest at 24–48 hours after the initial suckling. However, the level of No. 6 at the same time was 4.2 g/dl, which suggested

FIGURE 4 *Hematological observations of litter 5*



Remarks: f-Hb=Free hemoglobin concentration in plasma
II=Icterus index
DA=Direct agglutination test
IAT=Indirect antiglobulin test

that this piglet had received less colostrum than the others. In the piglets except No. 6, the maximum and minimum resistance in the erythrocyte osmotic fragility test markedly decreased 24 hours after the suckling. Only a slight decrease of the minimum resistance in the erythrocyte osmotic fragility test was seen in No. 6.

DISCUSSION

The clinical findings of hemolytic disease in neonatal piglets range from acute to relatively chronic types, and they vary among littermates[1,4].

GOODWIN & SAISON (1959)[13] reported on the variety of the symptoms of the dis-

ease; which ranged from symptomless to acute death. These variable clinical findings are considered to be due to the complete or incomplete antibodies and their antibody titers against the piglets' erythrocytes. According to GOODWIN & SAISON (1959)[13], piglets generally follow an acute course of the disease ending in death if the titer of the sow's incomplete antibody is greater than 1 : 512. ABE (1969)[2] reported that in cases showing a titer of maternal complete antibody less than 1 : 256, very mild clinical symptoms were seen, while in cases having a titer of 500-1,000, almost all of the piglets died with acute clinical symptoms.

In the present experiment, the authors studied 5 cases of the disease in which almost all the titers of complete and incomplete antibody in the dams were 1 : 0-1 : 32 and 1 : 4-1 : 64 respectively. These titers seemed to be very low and therefore incapable of causing typical hemolytic disease as was mentioned by GOODWIN & SAISON (1959)[13], and ABE (1969)[2]. It is well known that the agglutinin titer of colostrum to the erythrocytes of the newborn is higher than that of the maternal serum[15]. In our experiment, almost all of the dams' serum and their colostrum showed relatively low titers against the erythrocyte of the piglets.

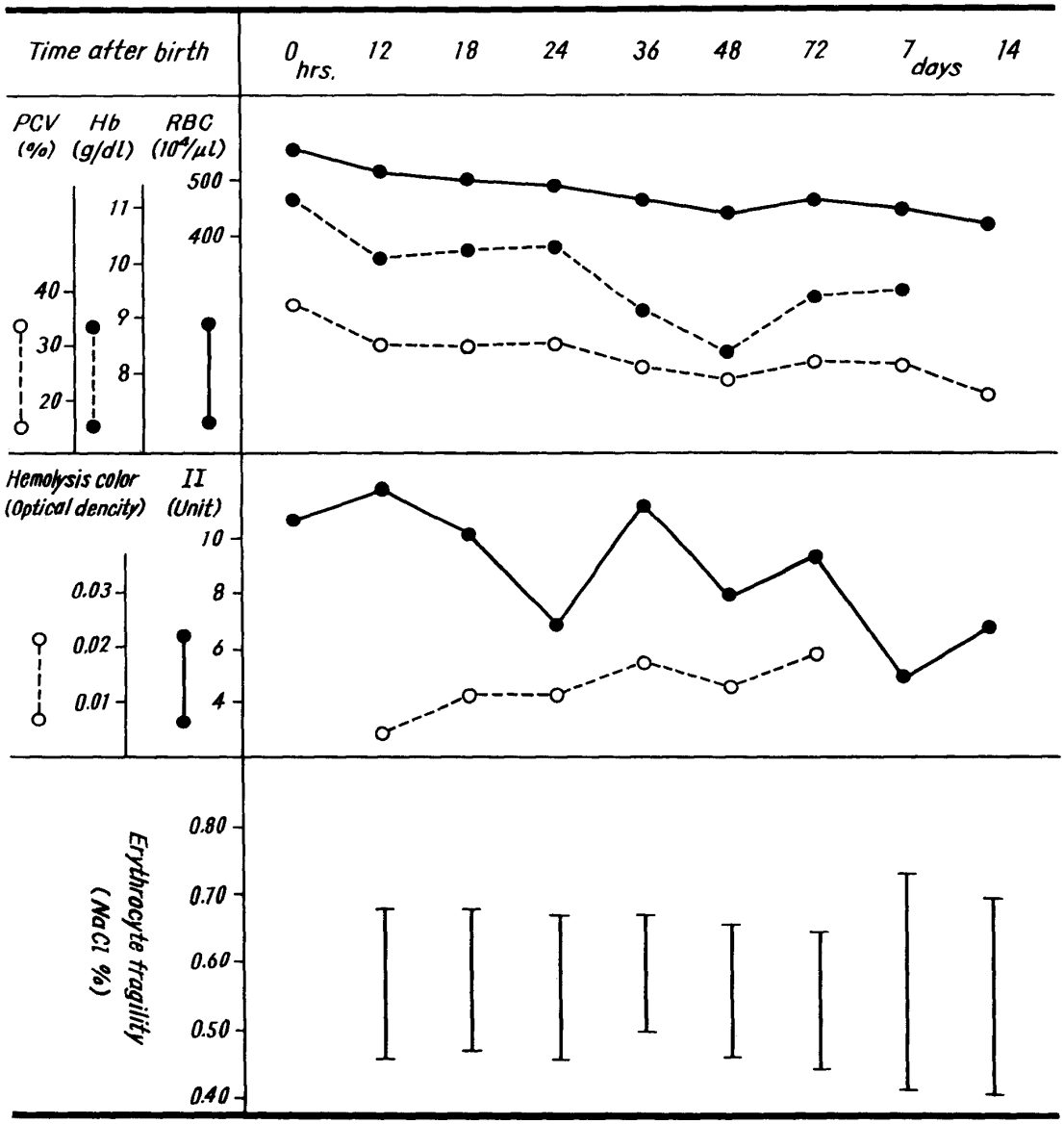
However, the titers of complete and incomplete antibody of the dam in litter 2 were 1 : 128 and 1 : 512 respectively to the erythrocytes of the offspring.

On the other hand, there was no case in which the dam possessed only incomplete antibody. For this reason, as mentioned previously it was thought that such cases would not develop hemolytic disease with clear clinical symptoms. In other words, these low titers of maternal antibody would be unable to produce clear icterus or hemolysis in the offspring. However, it should be emphasized that the fragility of the piglets' erythrocyte in almost all the cases showed extraordinary increases, whereas the level of free Hb concentration and the icterus index of the plasma increased only slightly. HUDSON (1955)[20] compared erythrocyte osmotic fragility between neonates and 7 month old pigs and found that the neonates showed weak resistance. However, the piglets employed in his studies showed enlargement of the liver and the spleen with icterus, which suggests it that the piglets suffered from subclinical hemolytic disease.

The authors determined the normal range of erythrocyte fragility in piglets derived from a dam which had no complete and incomplete antibody against her offsprings' erythrocytes. As shown in figure 5, the resistance range from minimum to maximum was 0.74-0.40%, this result was very similar to that obtained by LYON (1918)[25], ie., 0.70-0.45%. According to PERK et al. (1964)[30], about 85% of the red blood cells in newborn piglets are fetal types and their maximum resistance is 0.18%. This value is larger than that of adult swine, ie., 0.52-0.29%.

From these findings, the results of the erythrocyte osmotic fragility test in our cases can hardly be considered to be physiologically normal. Therefore, the erythrocyte osmotic fragility test should be very valuable method to detect the subclinical hemolitic

FIGURE 5 *Hematological changes in 4 healthy piglets from birth to 14 days old*



Remarks: II=Icterus index

disease. In such cases, decreasing of minimum resistance in the erythrocyte osmotic fragility test is evident and hemolysis may be seen in physiological saline. Consequently, as a practical method for the diagnosis of subclinical cases and for recognize the hemolysis, 1 or 2 drops of whole blood from neonates added to physiological saline might be very useful.

Subclinical symptoms may appear also if the ingested amount of colostrum, which

normally contains a relatively high concentration of antibody to the newborn's erythrocyte, is small. On the other hand, subclinical symptoms may also appear be weak when the amount of colostrum ingested is insufficient, and the piglets with the symptoms may consequently be diagnosed as having weak pig syndrome[23,32]. Therefore, it is advisable to carry out the erythrocyte osmotic fragility test in piglets which have been diagnosed as having weak piglet syndrome.

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