OBSERVATION ON THE DIFFERENTIAL BLOOD CELL COUNTS IN THE CIRCULATING BLOOD OF THE CHICK EMBRYO

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By

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Introduction

The development of the blood cells in the chick embryo has been studied by many investigators, who are inclined to draw their own conclusions which differ from each other (1, 3, 6). As for the different blood cells appearing in the circulating blood of the chick embryo, abundant literatures are available (1, 2, 3, 4, 6, 7). Above all, the different blood cell counts in the circulating blood were examined by Dawson (4), and the different blood cell counts in the yolk-sac blood of the early chick embryo was studied by Sugiyama (7). However, none of those workers reported on the blood cell counts in the circulating blood of the embryo. The present study was, therefore, designed to estimate the erythrocyte and leucocyte counts in the circulating blood of the chick embryo, and also to determine the changes in the differential blood cell counts of the chick embryo and the baby chick.

Materials and Methods

Chick embryos from three days of incubation were used, at an interval of 24 hours, up to the time of hatching. The baby chicks from one day to nine days after hatching were also used at the same interval. Besides, the chicks of 16 and 17 days after hatching were used. All of these materials were of the White Leghorn breed.

The circulating blood was collected from the heart of the specimens by inserting a glass capillary pippette designed by the authors into this organ. In younger embryos, sucking was necessary to obtain a full amount of the blood samples. The Büker-Türk counting chamber was used for the blood cell counting. To differentiate the erythrocytes and leucocytes, 1 per cent gentiana violet diluted 100 times by 0.85 per cent NaCl solution was used. The blood

samples of the embryo of from three days to eight days of incubation were directly transfered from the capillary pipette to the blood diluting pippette and were diluted 1:20. The samples from the embryos older than eight days of incubation and chick were diluted 1:10.

Smear preparations of the fresh blood were prepared in order to examine the changes in the differential blood cell counts during the development of the chick embryo. Thick smear preparations were made from the blood samples of the early embryos which contained relatively small number of the blood cells. The preparations were stained by the method of Amano *et al.* (2). Namely, they were fixed in 1 per cent chromic acid solution, rinsed in water, stained in the mixture of 0.04 g eosin, 0.04 g methylen blue, 0.01 g azur II in 270 cc distilled water for 60 minutes, rinsed again in water, and left for drying. Numbers of the erythrocytes in the estimation of the differential erythrocytes were 500 and 2000 in the early and later embryos, respectively. The leucocytes from 200 to 500 were also used for the observation.

Results and Discussion

A. The erythrocytes

1. Erythrocyte counts in the circulating blood

It has been known that the blood circulation of the chick embryo begins at 33 to 36 hours of incubation. In this investigation, however, the counting was made in the embryos older than 72 hours of incubation because of the difficulties encountered in collecting enough samples from those younger than this stage. The results obtained for the erythrocyte counts in the circulating blood are shown in Table 1 and Fig. 1.

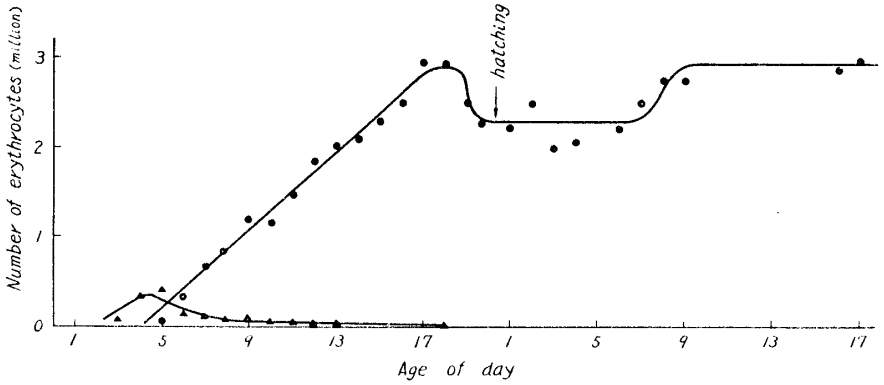

Fig. 1. Number of erythrocytes in the circulating blood of the chick embryos and baby chicks.
▲—— Primitive erythrocyte counts
●—— Definitive erythrocyte counts

The erythrocyte counts were about 90,000 in the average in the embryos at three days of incubation. The count showed a continuous increase during

the development of the embryo and the growth of the chick. In the embryos at 16 to 17 days of incubation, it was about 3,000,000 in the average. The value was equal to that obtained in the adult chicken. Therefore, it may be concluded that the erythrocyte counts of the embryo at 16 to 17 days of in-

Table 1. Differential erythrocyte counts in the circulating blood of the chick embryos and the baby chicks (average of three birds).

Age (day)	Erythrocyte counts	Primitive Erythrocyte					Definitive Erythrocyte				
		Megalobl. I	Proerythrobl. I	Erythrobl. I	Proerythrocy. I	Erythrocy. I	Hemacytobl. II	Proerthrobl. II	Erythrobl. II	Proerythrocy. II	Erythrocy. II
3*	90,000	0.4	2.2	97.4							
4	370,000		0.2	53.8	45.4	0.6					
5	500,000			0.8	82.2	2.4	0.2	12.0	2.4		
6	510,000				7.4	23.4		0.6	48.2	20.4	
7	780,000					16.0			48.0	36.0	
8	940,000					13.4		1.4	38.2	47.0	
9	1,300,000					8.0			1.5	52.0	38.5
10	1,120,000					4.8			5.4	49.0	40.8
11	1,460,000					5.0			5.2	32.3	57.5
12	1,830,000					3.4			12.8	32.6	51.2
13	1,990,000					1.0			1.8	30.0	67.8
14	2,080,000					1.0			0.4	24.4	72.8
15	2,300,000					0.6				6.8	92.5
16	2,470,000					0.4				4.6	95.0
17	2,930,000					0.2			0.2	1.6	98.2
18	2,910,000					0.2				1.0	98.8
19	2,470,000									1.6	98.4
20	2,290,000									1.8	98.2
1**	2,230,000										
2	2,480,000										
3	1,980,000										
4	2,160,000										
5	—										
6	2,220,000										
7	2,500,000										
8	2,750,000										
9	2,750,000										
16	2,860,000										
17	2,960,000										

Note; * days of incubation
** days after hatching

cubation reached to the normal count of the adult chicken. The count showed a rapid decrease in the embryo at 19 days of incubation, when it was 2,500,000. The level of the erythrocyte counts which has once reached at 16 to 17 days of incubation was not recovered until seven days after hatching. At eight to nine days after hatching, it was about 2,750,000, showing almost the normal level of the adult chicken.

It is well-known that, in the chick embryos, the yolk-sac respiration is switched to the lung respiration at one to two days before hatching or 19 days of incubation. The coincidence of the decrease of the erythrocyte counts with the change in the type of the respiration suggested the possible correlation between them. Moreover, it is most likely that the amount of the hemoglobin may decrease during from 19 days of incubation to seven days after hatching in which the erythrocytes decrease in number. A further study on this problem is expected by the present authors.

2. The differential erythrocyte counts in the circulating blood

The percentages of the different erythrocytes in the circulating blood of the embryos were estimated in the blood smear preparations of various stages of the embryos. The classification of the erythrocytes of Dawson (4) was used by the present authors, since they believed that his classifications is th best of all proposed by other investigators. The results are shown in Table 1.

The erythrocytes in the circulating blood of the embryos at three to four days of incubation exclusively consisted of the primitive erythrocytes (Figs. 3 and 4). No definitive erythrocytes were observed in these stages. Most of the primitive erythrocytes belonged to the erythroblast I (97.4%). The remainders are megaloblasts and proerythroblast I (Fig. 3). The definitive erythrocytes appeared in the circulating blood at five days of incubation (Fig. 5). They were easily distinguished from the primitive ones which were always larger in size, oval in shape and had delicate chromatins. The percentages of the definitive erythrocytes was 14.4 per cent of the total erythrocytes at five days of incubation. It was 69.2 and 84.0 per cent at six days and seven days of incubation, respectively. This indicated that, in the heart blood, the replacement of the primitive erythrocytes to the difinitive ones began to occur between five and six days of incubation. The primitive erythrocytes were still present in the circulating blood until 18 days of incubation, but they disappeared after 19 days of incubation (Fig. 6).

According to Dawson (4) who studied the appearance of the different erythrocytes in the heart blood of the chick embryo, the primitive erythrocytes appeared at three days of incubation and their replacement by the definitive erythrocytes takes place at the middle of the five days of incubation. He also stated that the primitive erythrocytes were present in the circulating blood at two weeks after hatching. On the other hand, Sugiyama (6) reported that

the replacement occurred around the middle of four days of incubation in the yolk-sac blood. In the present investigation, however, the definitive erythrocytes were not found in the embryos from three to four days of incubation. The present authors agree with Dawson on the time when the primitive erythrocytes were replaced by the definitive erythrocytes. The primitive erythrocytes, after 19 days of incubation was not found in the present investigation.

B. The leucocytes

1. Leucocyte counts in the circulating blood

The leucocyte counts were obtained in the embryos after nine days of incubation. The results are shown in Table 2 and Fig. 2.

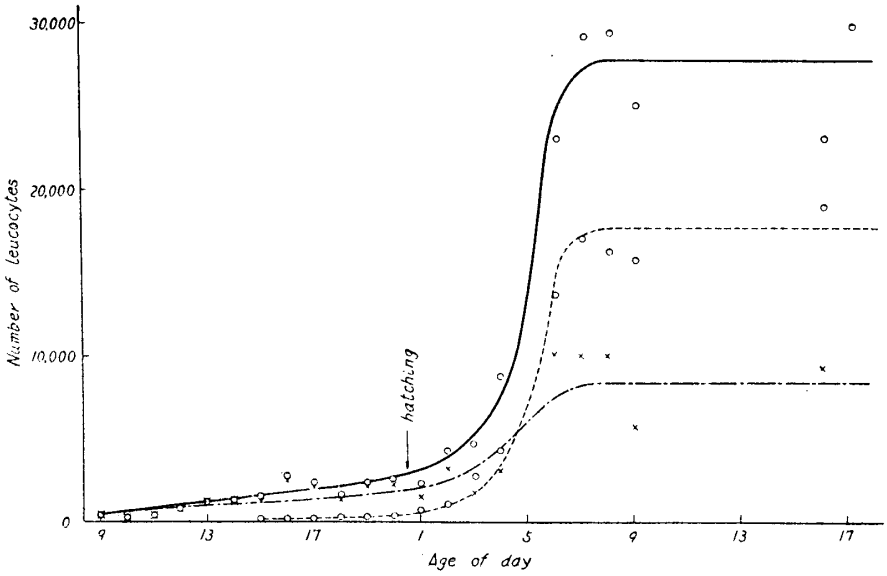

Fig. 2. Number of the leucocytes in the circulating blood of the chick embryos and the baby chicks.
○—— Total leucocyte counts
×----- Pseudoeosinophile counts
○----- Lymphocyte counts

The leucocyte counts at nine days of incubation were 400, which increased later to 1500 at 12 to 13 days of incubation. The count increased steadily in the older embryos, though it remained around 2500 during the embryonic stages. No remarkable changes in the leucocyte counts were found before and after hatching.

The leucocyte counts increased remakably during from four to six days after hatching, being approximately 30,000 at seven days after the hatching. They varied between 2,300 and 29,700 during from one to 17 days after hatching. Thus, the leucocyte counts reached the normal level at six to seven days after hatching.

2. Differential leucocyte counts

The results obtained for the percentages of the different leucocytes of the smeared circulating blood are shown in Table 2.

Table 2. Dfferential leucocyte counts in the circulating blood of the chick emblyos and the baby chicks (average of three birds).

Age (day)	Leucocyte counts	Pseudo-eosinophile (%)	Eosinophile (%)	Basophile (%)	Monocyte (%)	Lymphocyte (%)
9*	400	100				
10	300	100				
11	400	100				
12	1,500	100				
13	1,200	100				
14	1,300	96.0	2.0	2.0		
15	1,500	91.1	0.3	0.3		6.3
16	2,700	90.0	2.0	2.0		6.3
17	2,400	89.5	4.0	1.5		5.0
18	1,600	80.2	2.8	0.5	1.0	16.5
19	2,400	87.5	0.6	0.2	1.0	10.7
20	2,600	85.5	1.2	0.8	0.2	12.3
1**	2,300	66.5	0.7	0.3	2.0	30.5
2	4,300	73.5	0.2	1.5	0.8	24.0
3	4,700	36.0	0.7	3.3	3.0	57.0
4	8,800	36.2	0.7	8.1	6.2	48.8
5	—	—	—	—	—	—
6	26,000	39.3	0.5	2.3	5.5	52.4
7	29,100	34.5	0.5	2.0	4.5	58.5
8	29,300	35.7	1.5	1.1	5.9	55.9
9	25,000	23.0	1.0	6.9	7.0	63.0
16	30,000	31.0	1.0	1.5	3.3	63.2
17	29,700	—	—	—	—	—

Note ; * Days of incubation
** Days after hatching

Only pseudo-eosinophiles were present in the circulating blood in the embryo from nine to 13 days of incubation. The eosinophiles and basophiles appeared in the circulating blood in 14 days old embryos, occupying 2 per cent of the total leucocyte counts, respectively. The lymphocytes appeared at 15 days old embryos. Monocytes were found in 18 days old embryos. Generally, the pseudo-eosinopiles were dominant in the leucocytes of the circulating blood during the embryoic life. However, the lymphocyte counts showed a sudden increase in three days after hatching, and was more that 50 per cent of the total leucocyte counts. The differential leucocyte counts in the circulating blood of the baby chicks in three to four days after hatching become nearly

the same as those in the adult chickens.

The results in this study indicated that only the lymphocyte counts increased soon after the hatching (3 days), whereas the counts in the rest of the leucocytes, pseudo-eosinophiles, eosinophiles, basophiles and monocytes remained almost unchanged. Accordingly, the remarkable increase of the leucocyte counts of the circulating blood, at four to six days after the hatching, was probably due to the increase in the lymphocyte counts.

Roberts *et al.* (5) reported that the lymphocyte counts in the circulating blood was only 5 per cent, almost agreeing with the results in the present investigation.

Summary

The results obtained in this study may be summarized as follows;

1. The erythrocyte counts were 90,000 in three days chick embryos, increased during the embryonic development, and reached to the level of the adult chicken (approximately 3,000,000) in 16 to 17 days of the incubation. A temporary decrease in the count was observed around the time of the hatching, and they recovered to the previous level in eight to nine days after hatching.

2. The primitive erythrocytes in the circulating blood were partially replaced by the definitive erythrocytes in five to six days embryos. The former cells disappeared from the circulating blood just prior to hatching.

3. The leucocyte counts at nine days of incubation were relatively small during the embryonic life. They rapidly increased at six to seven days after hatching, reaching to the level of the adult chicken, approximately 30,000.

4. Most of the leucocytes in the circulating blood of the embryos were the pseudo-eosinophiles. The lymphocytes remarkably increased in three to four days after hatching.

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Explanation of Figures

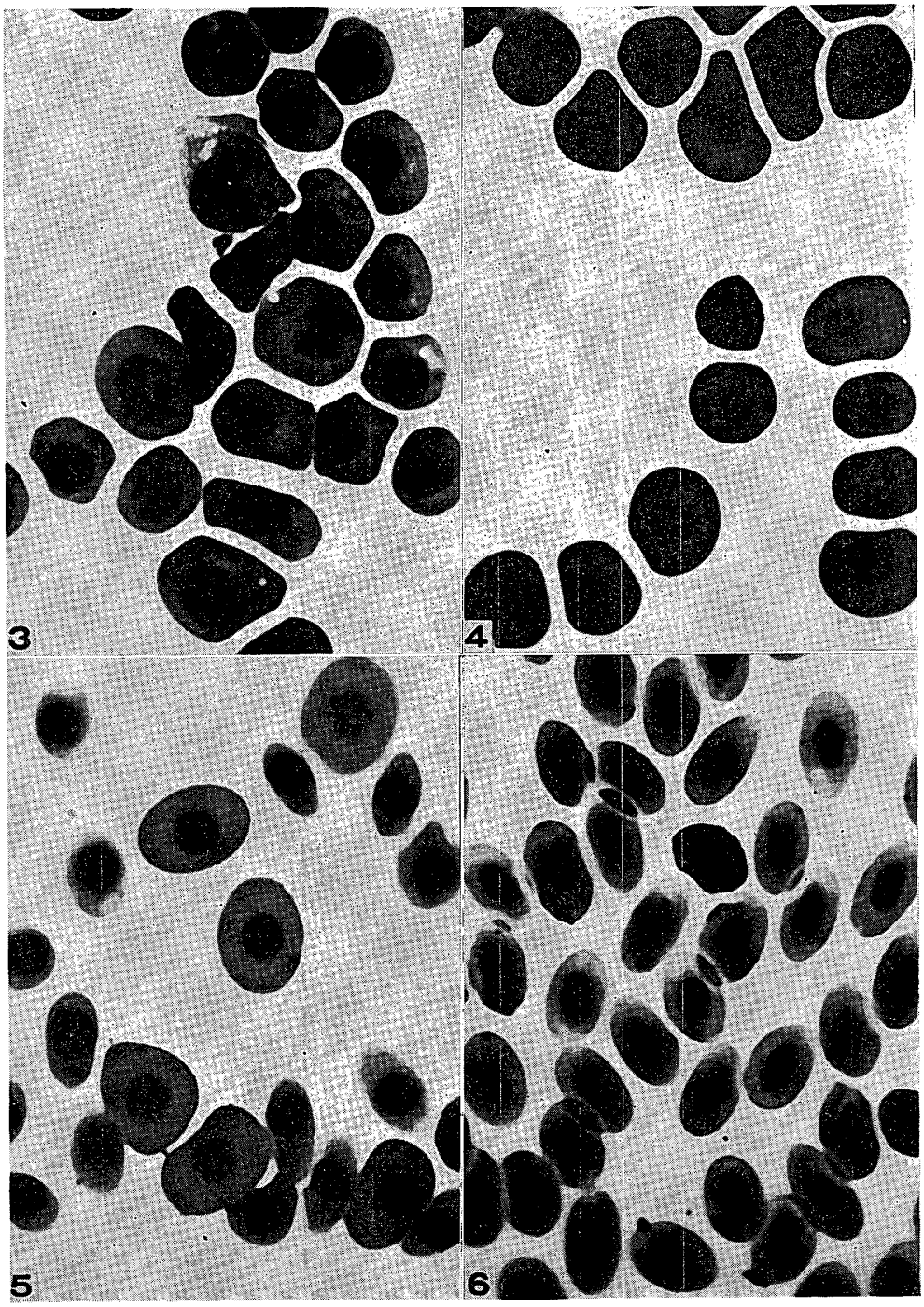
Successive stages of erythrocyte development in the circulating blood. Azur II-methylen blue-eosin stain (Amano et al.). ×1000.

Fig. 3. Megaloblast, proerythroblasts I and erythroblasts I from three days chick embryo.

Fig. 4. Primitive erythrocytes from four days chick embryo.

Fig. 5. Primitive and definitive erythrocytes from seven days chick embryo.

Fig. 6. Definitive erythrocytes from 19 days chick embryo.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