Studies on the Haemorrhagic Anæmia.

II. The Rate of the Oxidation and Reduction of the Reticulocyte.

By

Zunzaburo Nobuoka.

(信岡順三郎)

*(From the Institute of Physiology, Faculty of Medicine,
the Kyusyu Imperial University, Hukuoka,
Director: Prof. M. Itagaki.)*

INTRODUCTION.

Mathison¹⁾ succeeded by his own method in getting the curve, showing the rate of the oxidation and reduction of the blood, and concluded that the acids accelerated the rate of the reduction of the blood. Oinuma²⁾ devised a method of the measuring the rate of the oxidation and reduction. He also studied the influence of the temperature and the CO₂ upon it, and Kato³⁾ proved by means of a similar method that the alkali accelerated the rate of the oxidation and that at the same time it retarded the rate of the reduction of the blood.

There are only a few studies made with regard to the rate of the oxidation and reduction in the anæmic blood. Sano⁴⁾ recognized an acceleration of the rate of the reduction of the anæmic blood. Simidu⁵⁾ confirmed the finding of Sano and also added that the rate of the oxidation was retarded in the anæmic blood.

The present research was planned to investigate the rate of the oxidation and reduction of the reticulocyte, which remarkably increased in the blood of the animal suffering from haemorrhagic anæmia.

A part of this paper was read before the 10th Annual General Meeting of the Japanese Physiological Society on March 31st. 1931 in Nagasaki. Its brief description is to be found in the Jap. J. Med. Sci., III., Biophysics, 1931-33, 2, Proceedings 138.

A. *Method of Investigation.*

1. Experimental Material.

10 c.c. of the blood per kilo body weight was drawn daily from the vein of the ear lobe of rabbits. The anæmia was thus produced. The blood used for the present investigation was that of the 4th day when the anæmia advanced fairly.

The animal showed the maximum anæmia on the 5th or the 6th day of bleeding. The reason why we chose the blood of the 4th day was as follows.

The blood of the maximum anæmia absorbed a considerable amount of oxygen, so within a differential blood gas analysis apparatus of Barcroft, as it continuously absorbed oxygen within the system, sometimes the exact reading of the endpoint of the reaction was untenable. Although on the 4th day there began the consumption of oxygen by the blood, but it was very slight; even the number of the reticulocyte was rather few in comparing with that in the blood on the 5th day and after the 5th day, so we chose the 4th day blood. Even with this blood we sometimes met considerable difficulties in making an exact reading.

The blood drawn was defibrinated and by putting it into a centrifuge, the corpuscles were separated from the serum.

The blood corpuscles were washed three times with the physiological saline solution. The gruel of the blood corpuscles was divided into the upper and lower layers respectively. Each division was diluted three times with the physiological saline.

2. Method of Determining the Rate of the Oxidation and Reduction.

There are the methods of Mathison,¹⁾ Oinuma,²⁾ Kato³⁾ and Simidu⁵⁾. Their principles are about the same. Through the blood sample, which is contained in a long bubbling glass tube, bubbling at a uniform rate, for example, 15 or 30 per minute, a stream of the definite composition of oxidizing or reducing gas is sent in for a definite time. Then certain amount of the blood sample was drawn from the bubbling tube and the content of the oxygen was measured. By knowing the oxygen content after a definite time we can calculate the rate of the oxidation. I mainly adopted Oinuma's method, which was a modification of Mathison's. The bubbling tube was immersed in a warm bath at 38°C.

a. The rate of the reduction.

The blood was shaken in the air for full saturation with oxygen for 15 minutes just before the commencement of the experiment. For the reducing gas I used the compressed hydrogen gas, which was confirmed to contain 0.1% oxygen by analyzing it by means of Haldane's air analysis apparatus. The rate of stream was 60 or 80 bubblings per minute. (In Case 1, the rate of bubbling was 60 per minute and in the other cases it was 80).

b. The rate of the oxidation.

The blood was fully reduced prior to the oxidation experiment. 7 c.c. of the blood sample was put into a tonometer of about 250 c.c. capacity. The

tonometer was connected with a vacuum pump. First, the air in the tonometer was drawn, and by stopping the action of the pump the tonometer was shaken for 3 minutes; then the communication of the vacuum pump and the tonometer was established again, and by sucking the gas from the tonometer into the vacuum pump, the connection between the vacuum pump and the tonometer was again arrested, and the tonometer was shaken for 5 minutes. The connection of the tonometer with the vacuum pump was again established and thus all the gas from the blood was carried away by the vacuum pump. The blood sample was thus almost completely reduced.

For the oxidation of the blood sample I used a compressed air; the rate of bubbling was 100 or 120 per minute. (In Case 1. the bubblings were 120 and in the other cases they were 100).

c. The estimation of the percentage saturation of the blood with oxygen.

A certain time after the continuous bubbling, a portion of the blood sample was taken out of the bubbling tube and percentage saturation was measured. A Barcroft's big differential blood gas analysis apparatus⁶ was used. 2 c.c. of ammonia solution was put into each bottle and in the one side 1 c.c. of the blood sample was put under ammonia and in the bottle of the other side the same amount of the distilled water, free from air, was put. In both bottles a trace of saponine was added.

EXPERIMENTAL RESULTS.

In measuring both the rate of the oxidation as well as that of the reduction the estimation of oxygen contained in the blood sample was done after 5 minutes, 10 minutes and 15 minutes. The estimation of the oxygen contained after more than 15 minutes was not carried out, as we thought, in such an anaemic blood that consumed the oxygen of its own.

The results of experiment are arranged in tabular form in Cases 1-4.

Case 1. ♂

Days of bleeding	1	2	3	4	Blood corpuscles	Rate of reduction			Rate of oxidation		
						5'	10'	15'	5'	10'	15'
Body weight in kilo	3.05			2.93							
Blood drawn c.c.	31	28	30	46							
Haematocrite %	44	—	25	18							
Hb %	104	—	58	41							
					Lower layer	88.9	85.8	—	52.8	92.8	—
					Upper layer	79.8	67.6	54.9	20.8	53.8	—
						Reduction..... 60 bubbles per minute.					
						Oxidation 120 bubbles per minute.					

Case 2. ♀

Days of bleeding	1	2	3	4
Body weight in kilo	2.64			2.46
Blood drawn c.c.	26	38	10	43
Haematocrite %	43	33	23	17
Hb %	98	70	52	38

Blood corpuscles	Rate of reduction			Rate of oxidation		
	5'	10'	15'	5'	10'	15'
Lower layer	88.6	93.5	83.5	33.6	69.2	87.5
Upper layer	61.7	58.6	46.3	17.6	46.3	62.6

Reduction..... 80 bubbles per minute.

Oxidation100 bubbles per minute.

Case 3. ♀

Days of bleeding	1	2	3	4
Body weight in kilo	2.77			2.62
Blood drawn c.c.	28	28	26	40
Haematocrite %	42	34	27	20
Hb %	93	69	58	48

Blood corpuscles	Rate of reduction			Rate of oxidation		
	5'	10'	15'	5'	10'	15'
Lower layer	89.2	79.5	62.0	28.3	55.6	83.9
Upper layer	67.1	53.2	33.1	19.4	47.3	59.0

Reduction..... 80 bubbles per minute.

Oxidation100 bubbles per minute.

Case 4. ♂

Days of bleeding	1	2	3	4
Body weight in kilo	2.45			2.32
Blood drawn c.c.	26	27	25	40
Haematocrite %	48	31	23	16
Hb %	107	73	52	

Blood corpuscles	Rate of reduction			Rate of oxidation		
	5'	10'	15'	5'	10'	15'
Lower layer	88.1	82.7	74.8	25.5	50.8	72.4
Upper layer	62.1	52.8	36.7	20.6	36.8	52.6

Reduction..... 80 bubbles per minute.

Oxidation100 bubbles per minute.

CONCLUSION.

In looking over the result of the experiment, we conclude that the rate of the reduction is greater in the upper layer than in the lower layer, and the rate of the oxidation is greater in the lower layer. Therefore it seems that the reticulocytes are greater in the rate of the reduction and smaller in that of the oxidation than the adult erythrocytes, and besides that the rate of the reduction seems to exceed that of the oxidation.

The author thanks to Prof. M. Itagaki for his kind advice and criticism throughout this work.

REFERENCES.

- (1) Mathison, G. C.: J. Physiol., 1911, **43**, 347.
 - (2) Oinuma, S.: J. Physiol., 1911, **43**, 364.
 - (3) Kato, T.: Bioch. J., 1915, **9**, 393.
 - (4) Sano, T.: Zika Zassi, 1920, No. 244, 817.
 - (5) Simidu, K.: Jap. J. Med. Sci., II., Biochemistry, 1931, **2**, 267.
 - (6) Barcroft, J.: J. Physiol., 1908, **37**, 12, Barcroft, J. and Roberts, Ff. J. Physiol., 1910, **39**, 429.
-