THE LENGTH OF THE RETICULOCYTE CYCLE IN PIGEONS¹

DONA GAYLER GRAAM, Indiana State Teachers College

Introduction

In 1930 Eaton and Damron (1930 a and b) published two articles in which they purported to have demonstrated the average length of life of the rabbit's red corpuscles by counting the number of days between the peaks of reticulocyte cycles, the first cycle of which had been initiated by an artificial hemorrhage. My attention was called to the articles, and the experiment was repeated several times by myself and the reticulocyte cycles found to occur as they had stated. But the explanation that the distances between the peaks of the cycles represented the average length of life of the rabbit's red corpuscles could not be accepted, although there is a close relationship between the two. I have pointed out in another paper the reasons for not accepting this explanation.

When I was satisfied that the reticulocyte cycles occurred at regular intervals in the case of the rabbit, and that they were closely associated with the length of life of the red corpuscle, I undertook the same experiment in the pigeon. This animal was chosen because it had nucleated corpuscles in contrast to the non-nucleated corpuscles of the rabbit, and I wanted to compare the length of time between the cycles in the two animals. This report has to do with the length of time between the reticulocyte cycles in the pigeon.

Experimental Procedure

Since environmental factors, namely, food (Vaughn, Muller, and Zetzel, 1930, Muller, 1927 and 1929, a and b) and lowered temperature (Graam, 1934) may stimulate the haemopoietic function in the pigeon, I was very careful to make the environment as uniform as possible.

The pigeons were confined in cages large enough for them to walk about and were fed on a mixed grain diet (Vaughn, Muller, Zetzel, 1930) sometime before and during the experiments. The pigeons reported upon in this paper were held at room temperature, except as the temperature was markedly changed as a part of the experiment and the data included as a part of the notes of the experiment.

I discarded, as not fit for experimentation, pigeons that had too high a reticulocyte count, more than 10 or 12 per cent, and those that had too irregular reticulocyte counts, which could not be accounted for from the laboratory procedure.

The blood of the hemorrhage and the few drops of blood needed daily for staining, were obtained from the under wing veins by the puncture method.

¹ Part of the thesis required as part fulfillment toward the Ph.D. degree, Indiana University, 1933.

In order to facilitate the procedure of extracting a few drops of blood, the pigeon's feet were tied together. While the pigeon rested between the knees of the technician, its head was covered with the left hand while its left wing was held up with the same hand. By means of a needle the vein was pierced. The blood was drawn into an ordinary pipette which was inserted in the droplet that formed. After drawing two or three drops, the thumb of the left hand was placed over the wound to prevent further bleeding, while with the right hand the blood was immediately dropped into an equal amount of the staining solution, mixing the two by thoroughly shaking them. This must be done quickly to prevent clotting.

Hemorrhage was the chief method used to initiate the first of the reticulocyte cycles in each pigeon, but lowered temperature was also used to initiate reticulocyte cycles (Graam, 1934), and each method was found to be successful in initiating a reticulocyte cycle which was subsequently followed by similar but consecutively smaller cycles which occurred at regular intervals.

Staining Technique and Counting

A modification of the method used by Osgood and Wilhelm (1931) for the staining of human corpuscles was used and is explained in detail in a former paper (Graam, 1934). The counting was also explained in some detail in that article.

Rabbit	5	Ret. Cycle	Pigeon	Ret. Cyc	ele
No. 1		8 days	s No. 1		ys
No. 2		7 days	*No. 2	8 da	\mathbf{ys}
No. 3		8.5-9 days	*No. 3		\mathbf{ys}
No. 4		7.5-8 days	s No. 4	10 da	ys
No. 5		6.5-7 days	s Average	9.1-9.4 da	ys
Avera	ge7.	5-7.8 days	1		

Table 1. A comparison of the length of the reticulocyte cycle of the rabbit and the pigeon. The time between the reticulocyte cycles of the pigeon was, on an average, some two days longer than that between the retriculocyte cycles of the rabbit. *Pigeons No. 3 and No. 2 are shown in Figure No. 1 and in Figure No. 2.

Results

The reticulocyte response due to either hemorrhage or lowered temperature reaches its peak in from two and one-half to four days. Figure 1 shows pigeon No. 3 of Table 1, and is a clear case of a reticulocyte response to hemorrhage, reaching a peak formation between the twelfth and thirteenth days of the experiment and from two and one-half to three days after the hemorrhage. This initial reticulocyte peak is followed on the twenty-fourth and thirty-fifth days, by two successive reticulocyte peaks, at eleven-day intervals.

Figure 2 shows pigeon No. 2 of Table 1 and shows a reticulocyte response to hemorrhage, the cycle reaching its peak on the tenth day, three days after hemorrhage on the seventh day. A succession of reticulocyte cycles follow it. The reticulocyte peaks of the eighteenth and possibly of the twenty-sixty days follow the one due to hemorrhage on

15 - 49646



Fig. 1. Hemorrhage occurred on the tenth day after ten days of control, during which the reticulocytes remained uniform at 7 per cent. Two and one-half days after hemorrhage, between the twelfth and thirteenth days, the peak of the initial reticulocyte cycle occurred. This cycle was succeeded, at two eleven-day intervals, by reticulocyte peaks on the twenty-fourth and thirty-fifth days.



Fig. 2. Hemorrhage occurred on the seventh day after seven days of control, during which the reticulocyte percentage was approximately 5. Three days after hemorrhage on the seventh day, the peak of the initial reticulocyte cycle occurred on the tenth day. This cycle was succeeded at eight-day intervals by reticulocyte cycle peaks on the eighteenth and possibly the twenty-sixth days. On the tenth day (incidentally the day of reticulocyte cycle peak due to hemorrhagic stimulus), the environmental temperature was lowered. Four days later, on the fourteenth day, a peak of a reticulocyte cycle occurred. The variations of reticulocytes after the twenty-first day can scarcely be attributed to any previous reticulocyte cycles since their percentage does not vary far from that of the control period.

the tenth day and occur at eight-day intervals. There are at least three reasons why I believe the reticulocyte peak of the fourteenth and possibly of the twenty-second day to be initiated by a different stimulus than those occurring on the eighteenth and twenty-second days, which followed the hemorrhagic stimulus.

1. The temperature of the environment of this pigeon was considerably lower on the tenth day of the experiment, and four days later on the fourteenth day a reticulocyte peak was reached which I believe was initiated by the lowered environmental temperature (Graam, 1934).

2. Reticulocyte cycle peaks would be successfully lower if due to only one initial stimulus, but in this case *every other one* is successively lower.

3. In the several pigeons observed, the period between two successive reticulocyte peaks due to one stimulus has never been less than seven and one-half or eight days, but in this pigeon it was four days.

Therefore, the reticulocyte peaks occurring at four-day intervals are of two different orders, one series having been initiated by hemorrhage and the other series by lowered temperature.

Conclusions and Summary

1. The pigeon's haemopoietic mechanism reponds to a hemorrhagic stimulus as well as to a lowered temperature stimulus.

2. The initial reticulocyte cycles are followed by other cycles at regular intervals whether the initial one is induced by hemorrhage or by lowered temperature.

3. The intervals between successive reticulocyte cycles following an initial stimulus are approximately two days longer than the reticulocyte cycle intervals of a rabbit.

4. The reticulocyte cycle interval in the rabbit averages 7.5 to 7.8 days and averages 9.4 to 9.5 days in the case of the pigeon.

Acknowledgments

I am indebted to Dr. William Moenkhaus and Dr. Paul Harmon of Indiana University for their suggestions in obtaining data for this article. I take this opportunity to thank them.

Bibliography

Eaton, Paul and Damron, F. L.: The Southern Medical Journ., 23, 311. 1930a.

Eaton, Paul and Damron, F. L.: The Southern Medical Journ., 23, 311. 1930b.

Graam, Dona Gayler: Proc. Ind. Acad. Sci. 43, 201. 1934.

Muller, G. L.: Am. Journ. of Physiol. 82, 269. 1927.

Muller, G. L.: Am. Journ. of Physiol. 83, 130. 1929a.

Muller, G. L.: Am. Journ. of Physiol. 83, 259. 1929b.

Osgood, Edwin E. and Wilhelm, Mable M.: Proc. So. Exp. Biol. and Med. 29, 53. 1931.

Vaughn, J. M., Muller, G. L., and Zetzel, L.: Brit. Journ. Exp. Med. 11, 456. 1930.