

flow to such an extent that many colonies were on the verge of starvation during the summer months. Rainfall late in the summer resulted in a heavy flow of smartweed (heart's ease) nectar, enough in many cases to provide ample winter stores and in some cases a surplus was obtained.

State inspectors found but little European foulbrood but considerable sac brood and an enormous amount of American foul brood, the bulk being found in territory not worked by the inspectors for several years.

THE EFFECT OF LOWERED TEMPERATURE ON RETICULOCYTE FORMATION IN THE PIGEON

DONA GAYLER GRAAM, Indiana State Teachers College

INTRODUCTION

While studying increased reticulocyte formation in pigeons, due to hemorrhagic stimulus, certain observations were made concerning reticulocyte formation in pigeons, due to the stimulus of decreased temperature of the environment.

Many workers (Wills, 1932), (Vaughn, Muller, and Zetzel, 1930) have mentioned the extreme sensitiveness of the blood manufacturing mechanism of the pigeon to many factors. Muller (1927) (1929 a and b) has reported the effects on reticulocyte formation in pigeons to certain articles of food. In an unpublished article, the present writer has reported the effect of hemorrhage on reticulocyte formation in relation to the length of life of the red corpuscle in both the rabbit and pigeon. In the present report the effect of cold on the reticulocyte formation, in pigeons, is presented.

Experimental Procedure. The results of this report are based on two planned experiments and two accidental observations. Two pigeons were purposely kept under uniform environmental conditions for nine months, then exposed to a rapid fall in the environmental temperature. Two accidental observations were made on two other pigeons, each of which at different times experienced a rapid fall in their environmental temperature.

In all four pigeons studied the reticulocyte count and environmental temperature were recorded. In case of pigeons of the planned experiments, the body temperature was also recorded over the entire experimental period and the reticulocyte count and body temperature were taken at the same time every day except the day the pigeons were exposed to cold; then their body temperatures were taken by way of the cloaca at one hour intervals for six consecutive hours. This article is a report of the results in the case of the two pigeons used in the planned experiments.

Staining Technique and Counting. I found the best method for staining the new red blood cells of the pigeon, referred to as reticulocytes in this paper, to be a modification of the method of Osgood and Wilhelm (1931) for the staining of human reticulocytes.

The staining solution consisted of 1 per cent aqueous solution (1 gr. —100 gr.) of Brilliant Cresyl Blue, .85 per cent NaCl and .3 per cent sodium citrate. Six drops of this solution were used to two or not more than three drops of the fresh blood. The mixture of blood and staining solution was shaken thoroughly and allowed to stand from 10 minutes to half an hour. The difference in time made no difference in the reticulocyte per cent, but if the mixture stood longer, the corpuscles began to disintegrate.

A droplet of the stained blood mixture was removed to a microscope slide, spread and allowed to dry. The red cells were examined immediately. The oil was dropped directly on the dried smear. If desired, the preparation can be kept for several weeks or months by mounting in balsam under a cover slip. However, the slide must be kept in the dark or it will soon fade. It is not necessary to counterstain.

Counting. Practically every red cell of the pigeon's blood contains some "basophilic" substance in the protoplasm. The younger cells contain the most of this so-called "basophilic" substance. One must learn to be consistent and he must arbitrarily decide which of these cells to call reticulocytes. I arbitrarily called those cells reticulocytes which had a complete chain of basophilic substance around the nucleus.

All those cells in one field of the microscope were counted (a micrometer disk was used to facilitate matters), the number set down and the field gone over a second time in order to count the reticulocytes. The slide was then moved to an adjacent field and the cells counted as above. Five hundred or more cells were counted in this way and then the percentage of new cells determined.

Food. Vaughn, Muller, and Zetzel (1930) found that after feeding pigeons a standard mixed grain diet for several weeks or even months, an established level of near 10 to 12 per cent of reticulocyte production was established. If this diet were modified by other substances such as meat or liver extract an immediate rise in reticulocyte manufacture was produced.

The pigeons used in all my experiments were grain-fed pigeons and the reticulocyte counts were extremely low and of great uniformity of number during the control periods.

RESULTS

Two pigeons, one of which is represented in figure 1 and the second in figure 2, were examined for reticulocyte percentage during a control period of five days immediately before they were placed outside in a temperature 40°-50° F. colder than room temperature. The response of the two pigeons was very similar as can be seen in figure 1 and figure 2. The temperature of the room was fairly uniform during the control period at about 72° F. and the reticulocyte count varied only slightly at this temperature.

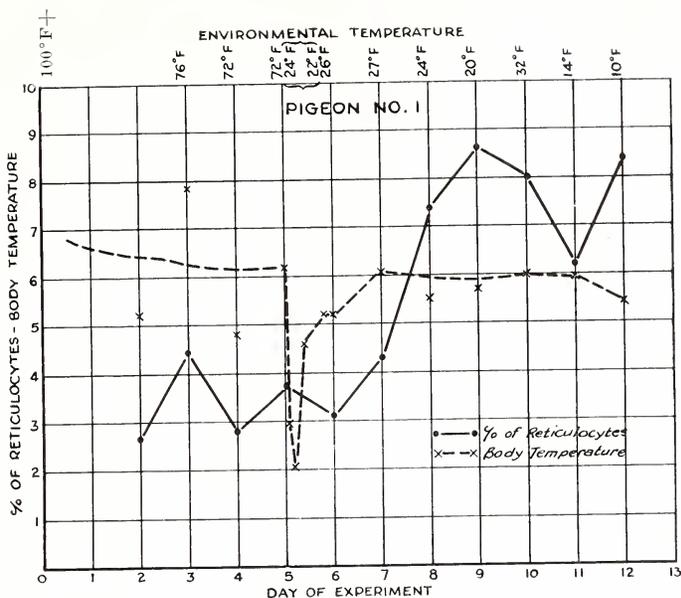


Fig. 1. The environmental temperature, the body temperature and the reticulocyte percentage were taken every day of the experiment and on the fifth day, after exposure to a fall in environmental temperature of between 40° - 50° F., the body temperature was taken several times. The body temperature fell rapidly at this lower temperature, but after twenty-four hours the body temperature was back to normal. A reticulocyte peak was noted on the fourth day after the pigeon experienced the lower environmental temperature on the ninth day of the experiment. The reticulocyte response to cold continued from the eighth day to the end of the experiment on the twelfth day.

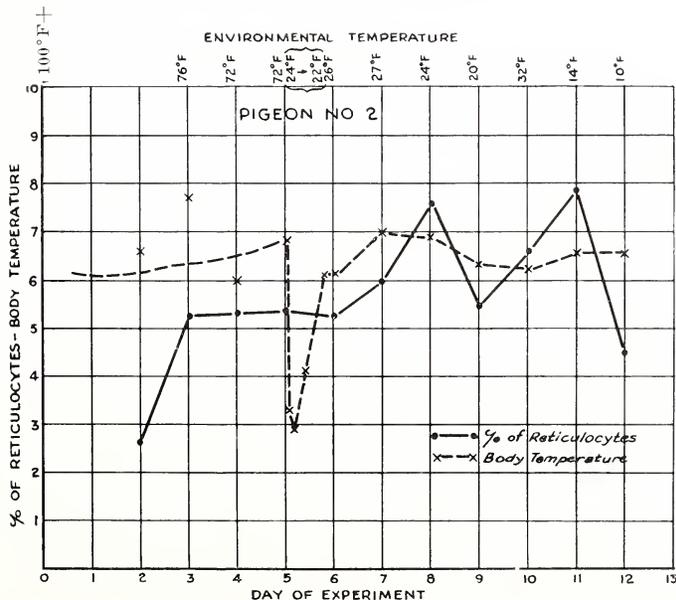


Fig. 2. Same explanation as for Figure 1 except the reticulocyte peak was reached on the third day after the pigeon experienced the lower environmental temperature or the 11th day of the experiment. The reticulocyte response to cold continued from the seventh day to the end of the experiment on the twelfth day.

On the fifth day of the experiment, the pigeons were placed in a colder temperature of 24° F. and the temperature remained at that level for the rest of the experiment. The body temperature of each was recorded every hour for six consecutive hours after exposure to cold and thereafter at intervals of twenty-four hours. It will be seen that there was a distinct fall of 4° or 5° F. in body temperature on exposure to cold in both cases but on the morning of the sixth day, twenty-four hours after exposure to cold, the body temperature had again reached normal or a matter of 105.3° F.; 106.1° F. respectively. From the sixth day on to the twelfth day, at which time the experiment ended, the temperature of the pigeons remained near this normal level. The reticulocyte count was still above that of the control period, on the twelfth day of the experiment. The reticulocyte percentage increased and reached a peak three to four days after exposure to cold and lowering of body temperature.

The reticulocyte response to hemorrhage which I have explained in an unpublished article and the reticulocyte response to cold are very similar, but if the cold produces an intravascular destruction of red corpuscles and so initiates the response in reticulocytes, I am not prepared to say.

CONCLUSIONS AND SUMMARY

1. Lowering the environmental temperature as much as 40° F. causes the body temperature of pigeons to fall a maximum of 4° F. in five hours.
2. The pigeons recovered their normal temperature of 105° F. in less than twenty-four hours.
3. A peak of reticulocyte formation occurred on the fourth day (three days in some pigeons) after the pigeon's exposure to cold.
4. Presumably, the haemopoietic mechanism of the pigeon responded to a "cold" stimulus in a way similar to its response to a hemorrhage stimulus.

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