ON THE RELATION OF THE CATALYTIC ACTIVITY OF THE BLOOD TO THE NUMBER OF RED BLOOD CELLS IN HEALTH, AND TO THE NUMBER OF WHITE BLOOD CELLS AND THE BODY TEMPERATURE IN PERTONITIS.\textsuperscript{1}

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(From the Pathological Laboratory of the Johns Hopkins University.)

Before entering into the discussion of the main object of this paper, namely, the relation of the catalytic activity of the blood to the white blood cells and the body temperature in peritonitis, a heretofore obscure finding might be explained to a certain degree, at least.

It will be remembered\textsuperscript{2} that the catalytic activity of the blood of a normal rabbit is practically constant from day to day, while the activity of the blood of different rabbits may vary within relatively wide limits. This phenomenon seemed unexplainable except for the fact that in rabbits of the same litter and the same grade of development there was a greater correspondence of the activity of the blood than in those where there was a visible difference in size and age. The relation of the catalytic activity of the blood to the number of red blood cells may, as will be seen, explain these facts.

THE RELATION OF THE CATALYTIC ACTIVITY OF THE BLOOD TO THE RED BLOOD CELL COUNT.

Bergengrün was able to show as early as 1888 that the power of the blood to decompose hydrogen peroxide depended almost entirely upon the red blood cells, and while the white blood cells and the blood serum are also capable of splitting hydrogen peroxide, their action is so slight that it is hardly to be taken into consideration when compared to the great activity which the red blood cells exhibit. This work has been repeatedly confirmed, and, needless to say, if

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\textsuperscript{2}Winternitz, \textit{Jour. of Exp. Med.}, 1909, xi, 200.
any practical value is to come from the determination of the cata-
lytic activity of the blood, its relation to the number of red blood
cells, which apparently are the source of the blood's action, must
be known both in health and in disease.

We have selected a number of healthy rabbits and carefully deter-
mimed their red blood count and the power of their blood to decom-
posse hydrogen peroxide. In order that the counts should be of
greater value, we removed five cubic centimeters of blood from the
ear vein of each rabbit and placed this in a graduated centrifuge
tube with ten cubic centimeters of sodium citrate solution. The
tubes were then centrifuged for a definite length of time and the
volume of the red blood cells noted. In the table given below, we
have recorded for comparison the volume of red blood cells, the red
blood count and the catalytic action of the blood in each case. The
table represents three series of experiments, and in calculating the
relation of volume to number of red blood cells, it should be remem-
bered that only the members of the same series are comparable, as
they represent the corpuscles which were centrifuged for the same
length of time.

<table>
<thead>
<tr>
<th>No.</th>
<th>Red blood count</th>
<th>Vol. of R. B. C. in 5 c.c. of blood</th>
<th>Catalytic activity of blood (1-200 dilution.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10s  30  45  60  75  90  120</td>
</tr>
<tr>
<td>1</td>
<td>5,160,000</td>
<td>1.8 c.c.</td>
<td>20.6 26 29.8 33 35.6 38.2 40.4 42.2</td>
</tr>
<tr>
<td>2</td>
<td>4,870,000</td>
<td>1.7</td>
<td>20.8 26.4 30 33.2 35.2 39 41.8 43.6</td>
</tr>
<tr>
<td>3</td>
<td>6,292,000</td>
<td>2.2</td>
<td>22.6 28.8 44.6 47.6 50.2 57.4 52.4</td>
</tr>
<tr>
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<td>1.62</td>
<td>20.4 25.8 28.8 32 35.2 37.4 40.4 43</td>
</tr>
<tr>
<td>5</td>
<td>4,192,000</td>
<td>1.45</td>
<td>20.4 25.2 29 32.8 35.6 39.2 42.4 45</td>
</tr>
<tr>
<td>6</td>
<td>5,320,000</td>
<td>1.95</td>
<td>24 31.4 37 42 46 49 51.6 53.2</td>
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</table>

Second series.

<table>
<thead>
<tr>
<th>No.</th>
<th>Red blood count</th>
<th>Vol. of R. B. C. in 5 c.c. of blood</th>
<th>Catalytic activity of blood (1-200 dilution.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10s  30  45  60  75  90  120</td>
</tr>
<tr>
<td>1</td>
<td>4,488,000</td>
<td>1.7 c.c.</td>
<td>17.6 22.4 25.6 28 30.8 33.2</td>
</tr>
<tr>
<td>2</td>
<td>6,118,000</td>
<td>2.4</td>
<td>20 24.4 28 31 34 36.4</td>
</tr>
<tr>
<td>3</td>
<td>4,000,000</td>
<td>1.7</td>
<td>18.8 22.8 26.6 29.8 32.6 35.4</td>
</tr>
<tr>
<td>4</td>
<td>5,416,000</td>
<td>2.15</td>
<td>19.8 24.2 28.6 32 35.4 38</td>
</tr>
</tbody>
</table>

Third series.

<table>
<thead>
<tr>
<th>No.</th>
<th>Red blood count</th>
<th>Vol. of R. B. C. in 5 c.c. of blood</th>
<th>Catalytic activity of blood (1-200 dilution.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10s  30  45  60  75  90  120</td>
</tr>
<tr>
<td>1</td>
<td>4,760,000</td>
<td>1.82 c.c.</td>
<td>24.6 31.6 37 42 45.4 47.6</td>
</tr>
<tr>
<td>2</td>
<td>5,314,000</td>
<td>2.00</td>
<td>24.6 31.4 37 41.8 45.2 47.2</td>
</tr>
<tr>
<td>3</td>
<td>4,364,000</td>
<td>1.65</td>
<td>19.4 24.8 29.6 33.6 37 39.6</td>
</tr>
<tr>
<td>4</td>
<td>2,356,000</td>
<td>0.96</td>
<td>14.4 17.8 20.8 23.8 26.4 28.4</td>
</tr>
</tbody>
</table>

It will be seen at a glance from the above table that there is, as
has been frequently shown, a definite relation between the volume
and number of red blood cells. On the other hand, while there is
a definite relation between the catalase and the red blood cells, these
do not correspond exactly; for example, in Series 2, with a high
blood count of over six millions, one would expect the catalytic
activity to be very great, which, however, is not the case. It is

only fair to say, then, that in most cases a very high blood count
is accompanied by a high catalytic action and vice versa; lesser dif-
fferences in the blood count do not appear to be of equal importance
in varying the intensity of the action of the blood towards hydrogen
peroxide.

We have attempted to arrive at the relation of the red blood
count and the catalytic activity of the blood by still another method.
After determining both of these factors for a healthy rabbit, the ani-
mal was bled four times in relatively rapid succession and the blood
count and the catalytic activity determined after each bleeding.
Number of Red Blood Cells in Health.

The first five cubic centimeters of each bleeding were caught in sodium citrate solution and later centrifuged in graduated tubes to determine the volume of corpuscles. The results are expressed in the following table:

<table>
<thead>
<tr>
<th>Time</th>
<th>Number of R. B. C.</th>
<th>Vol. in c.c.</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.30 a.m.</td>
<td>3,400,000</td>
<td>1.15</td>
<td>17</td>
<td>21.2</td>
<td>25.2</td>
<td>28.4</td>
</tr>
<tr>
<td>10.45 a.m.</td>
<td>2,640,000</td>
<td>1.15</td>
<td>17</td>
<td>21.2</td>
<td>25.2</td>
<td>28.4</td>
</tr>
<tr>
<td>11.30 a.m.</td>
<td>1,888,000</td>
<td>1.15</td>
<td>17</td>
<td>21.2</td>
<td>25.2</td>
<td>28.4</td>
</tr>
<tr>
<td>11.45 a.m.</td>
<td>1,712,000</td>
<td>0.8</td>
<td>17</td>
<td>21.2</td>
<td>25.2</td>
<td>28.4</td>
</tr>
<tr>
<td>12.30 p.m.</td>
<td>1,416,000</td>
<td>0.6</td>
<td>17</td>
<td>21.2</td>
<td>25.2</td>
<td>28.4</td>
</tr>
<tr>
<td>12.45 p.m.</td>
<td>1,392,000</td>
<td>0.6</td>
<td>17</td>
<td>21.2</td>
<td>25.2</td>
<td>28.4</td>
</tr>
</tbody>
</table>

After the volume of corpuscles had been determined, half a cubic centimeter of corpuscles was removed from each tube and a 1 to 800 dilution made in distilled water. Five cubic centimeters of these solutions were then determined for their catalytic power and, as will be seen from the table below, the activity of the corpuscles removed was the same in each case.

Red Blood Corpuscles in Dilution 1-800.

<table>
<thead>
<tr>
<th>Bleeding</th>
<th>Vol. of R. B. C.</th>
<th>15</th>
<th>20</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.15</td>
<td>42</td>
<td>50</td>
<td>50.8</td>
<td>51.2</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>41.6</td>
<td>50</td>
<td>50.4</td>
<td>50.6</td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
<td>41</td>
<td>49.4</td>
<td>50.6</td>
<td>50.8</td>
</tr>
<tr>
<td>4</td>
<td>0.6</td>
<td>41.2</td>
<td>49.8</td>
<td>50.3</td>
<td>50.6</td>
</tr>
</tbody>
</table>

The intimate relation between the catalytic activity of the blood and the number and volume of red blood cells is clearly emphasized in the above experiments. While the number of red blood cells varies directly with the catalytic action of the blood in different animals, the variation is not always proportional, and so in the same animal the curve of the catalytic activity will depend greatly on the number of corpuscles, even though the decrease in the power of the blood to split hydrogen peroxide may not be as great as the decrease in the number of red blood cells after bleeding.

The problem, therefore, which confronted us in the beginning of this work, namely, the variations found in the catalytic activity of the blood of healthy rabbits, seems somewhat clearer. Large healthy animals are likely to have a high red blood content, and also...
M. C. Winternitz and J. P. Pratt.

a high catalytic activity. Animals of the same litter are likely to be of the same general state of development, and, therefore, having about the same red blood count will probably have about the same power of decomposing hydrogen peroxide in their blood.

It still remains for us to determine the relation of the red blood count to the variations found in the catalytic activity of the blood in disease, and experiments with this point in view are now being carried on.

In a previous article, it was shown that as a result of experimentally produced peritonitis in rabbits, whether the latter was brought about by bacteria or as a result of extravasation of irritant substances like urine, there was a rise in the catalytic activity of the circulating blood as determined by the method employed. It was shown that this rise was definitely determinable within two hours after the bacteria had been introduced into the peritoneal cavity, and that the catalytic activity of the blood steadily increased, reaching its maximum in some cases within six hours from the beginning of the experiment, while in others it remained excessively high for more than thirty-six hours from the time the peritonitis had commenced.

It occurred to us at that time that the determination of the catalytic activity of the blood might be of value clinically in determining the onset of serous cavity infections, and especially the peritonitis in cases of typhoid fever, appendix rupture, etc. Work is now being done with this point in view in the wards of the Johns Hopkins Hospital and will be reported in detail later.

In the course of our work this year, we have had numerous opportunities of confirming the results previously obtained on animals. We have studied many cases of infected rabbits with this point in view, but more interesting possibly than these are several cases in which as a result of some operative procedure where an entirely different result was sought, a peritonitis ensued. It may be of interest to cite one of these cases in detail.

Rabbit No. 57.—Spleenectomy. The operation was apparently satisfactory, and the animal recovered promptly. It was slightly drowsy on the second day, but this would have caused no particular anxiety if there had not been such a rapid and marked rise in the catalase of the blood. We have learned that this

*Jour. of Exper. Med., 1909, xi, 120.
is not what is to be expected following splenectomy. This rise continued for
several days, as shown by the chart, even though the animal did not seem
particularly ill. Its general condition became worse and on April 23 a tumor,
which had been present when the animal was admitted, was explored. The
mass lay in the subcutaneous tissue of the left back, and on incision a large
encapsulated mass of very foul smelling pus was revealed.

At autopsy the animal was much emaciated. The abdomen contained a
small amount of blood-stained fluid. Over the peritoneal surfaces there was
a slight friable exudate of grayish material which in places bled on removal.
The exudate was most abundant in the upper portion of the abdomen just
beneath the operative incision. Several of the sutures had sloughed, and here
the bowel was glued to the parietal peritoneum. Microscopically, the exudate
was composed of fibrin and leucocytes; the latter, however, were poorly pre-
served. This exudate showed everywhere an invasion by capillaries about
which numerous young fibroblasts and round cells could be seen.

**CHART 2.** Showing curve of activity during first fifteen seconds.

It will be seen from Chart 2 that with the peritonitis there was a
rise in the catalytic activity of the blood, and this was the first indi-
cation that the animal was ill, since clinically it showed practically
no untoward signs. This case suffices to emphasize the change in the catalytic activity of the blood following peritonitis, and furthermore shows that it may be the first sign of such infection in animals.

The work to be reported was undertaken with the hope of determining the relation of the catalytic activity of the blood to the body temperature and to the number of white blood cells.

THE RELATION OF THE CATALYTIC ACTIVITY OF THE BLOOD TO THE BODY TEMPERATURE AND THE NUMBER OF WHITE BLOOD CELLS.

As is well known, the body temperature of normal rabbits varies within wide limits, the normal ranging between 98° F. and 102° F.
Number of Red Blood Cells in Health.

This variation has no effect upon the catalytic activity of the blood, for it has been previously shown that the catalase of a single animal's blood is constant from day to day,

In the first series of experiments concerning the relation of the body temperature and number of white blood cells to the catalytic activity of the blood, it was attempted to produce a hyperpyrexia alone. This was accomplished by puncturing the heat regulating center described by Aronsohn and Sachs which they located in the anterior medial portion of the corpus striatum. We are indebted to Dr. W. G. MacCallum who kindly performed this operation for us in several cases.

Chart 3 represents the catalytic activity of the blood as determined in the first fifteen seconds (hollow dots) and its relation to the body temperature (solid dots) and white blood cells (crosses).

*Arch. f. d. ges. Physiol., 1885, xxxvii, 232.*
Chart 3 illustrates clearly that while there may be a marked hyperpyrexia resulting from a puncture of the anterior portion of the corpus striatum there is no corresponding change in the catalytic activity of the blood or the leucocyte count.

The two following experiments illustrate further the entire independence of the body temperature, the catalytic activity of the blood, and the leucocyte count.

Rabbit No. 10.—In this case, a peritonitis was caused by the introduction of potato shavings saturated with a culture of Staphylococcus aureus. The animal lived four days. At autopsy the abdominal cavity contained a considerable amount of fibrino-purulent exudate, the fibrinous portion being most abundant about the potato shavings. Throughout the viscera there were many miliary abscesses to be made out.
Chart 4 illustrates that even though there may be a decided fall in the body temperature and the leucocyte count, the catalytic activity of the blood may rise above normal.

Rabbit No. 10.—Here the peritonitis was caused by ligating the distal end of one ureter and slitting this proximal to the ligature so that the urine would drain into the abdominal cavity. The animal lived for forty-three hours, when it was sacrificed. The autopsy showed an extensive fibrino-serous peritonitis.

In this case (see Chart 5) there is a rise in the leucocytes together with the rise in the catalase of the blood, while the body temperature shows a decided fall.

Rabbit No. 15.—In this case a small portion of aleuronat was suspended in normal saline solution, and injected into the left pleural cavity. The animal was only slightly ill the next day and rapidly recovered as far as could be judged from external appearances. Several days later he received a second injection which proved fatal.

In Chart 6 there is no change in the catalytic activity of the
blood but a rise in both the body temperature and the white blood cells.

CONCLUSIONS.

The catalytic activity of the blood of normal rabbits varies almost directly with the volume and number of red blood cells. This explains to a certain extent at least why animals of the same general degree of nutrition, and of the same litter, should have about the same activity since they are likely to have the same number of red blood cells, and why healthy large animals should read high while small poorly nourished ones should read low.

Accompanying the hyperpyrexia resulting from puncture of the corpus striatum of a rabbit's brain, there is no change in either the catalytic activity of the blood or the white blood count. In experimentally produced peritonitis, the catalytic activity of the blood always rises, and is, therefore, absolutely independent of body temperature and white blood cells since one or both of these may rise, fall or remain stationary while the catalytic action increases.