THE OXYGEN AFFINITY OF HAEMOGLOBIN IN SPLENECTOMIZED BULLFROGS

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(With One Text-figure)

EVIDENCE obtained from previous studies suggests that one of the factors which influence the affinity of amphibian haemoglobin for oxygen is the locus of erythrocyte formation (Barcroft, 1934; Hall, 1934). It has been shown for the developing bullfrog (McCutcheon, 1936), and also for different species of the class Amphibia (McCutcheon & Hall, 1937), that the oxygen dissociation curve shifts from left to right depending upon the extent to which the specimen is aquatic, semiaquatic, or terrestrial.

It has previously been shown by Jordan & Speidel (1923 a, 1923 b) that there are three successive haemopoietic loci in the frog; viz. kidney, spleen, and bone marrow, and that the spleen and bone marrow may alternate as erythrocyte sources in the adult. Apparently the bone marrow is the primary source only during periods of greatest metabolic activity (spring of the year); at other times the spleen is the primary source. They found that if they splenectomized a frog and allowed sufficient time to elapse, the haemopoietic locus would be changed either back to the kidney or to the bone marrow. However, only a small number of cases shifted to the kidney.

In view of the possible importance of the various loci of erythrocyte formation on the oxygen affinity of haemoglobin it seemed likely that a study of the haemoglobin from splenectomized frogs might help to evaluate the influence of this factor.

MATERIAL AND METHOD

The bullfrog, *Rana catesbiana* Shaw, was the experimental animal for this investigation. The specimens were kept in a tank with a sloping bottom. The tank was partially filled with water which was changed daily. The frogs were fed fresh pork and beef liver by forcibly starting strips of the meat down the throat. This appeared to be satisfactory, for the frogs recovered rapidly from operations and seemed quite normal in every way.

Spleens were removed through an incision in the ventral abdominal wall. The vessels supplying the spleen were first ligated, the spleen was removed and dropped immediately into distilled water, and then the incision was closed with silk thread.

1 This work was done in the Zoological Laboratories at Duke University, Durham, North Carolina.
Peritoneum and musculature were sutured first, and then the integument. Light ether anaesthesia was used. The first few frogs failed to recover because the sutures were too tight and pulled out, or too much ether was used. When these factors were corrected, recovery was complete in every case and the wounds healed perfectly.

The oxygen dissociation curves of the haemoglobin were determined by the use of a spectrocomparator (Hall, 1935) according to a modified technique for small quantities of blood described elsewhere (McCutcheon, 1936). Curves were determined for a solution obtained by maceration of the spleen as soon as it was removed from the frog. It was originally intended to use this as the standard for each frog, but for reasons to be mentioned later it was found necessary to take a sample from the heart at the time of splenectomy.

A sample of about 0.5 c.c. was taken from the heart with a syringe at the time of splenectomy and again at the end of each experiment. A time approaching 100 days intervened between the initial and the final sample—a period which approximates the life of a frog erythrocyte (Jordan & Speidel, 1925). All dissociation curves were determined for haemoglobin solutions with an oxygen capacity of 0.2 vol. % or less, in M/30 phosphate buffered solution at pH 7.38, with temperature controlled at 25.3°C.

RESULTS

It was originally intended to use a haemoglobin solution from the macerated spleen to determine the oxygen affinity of the haemoglobin at the time of splenectomy. This would minimize the operative procedure. However, a number of difficulties made it necessary to take a sample from the heart also at this time. The solution obtained from the spleen was sufficient for only two or three points on the curve; it was occasionally necessary to use small amounts of saponin to obtain haemoglobin without too great dilution, and the solution was often somewhat murky, which made the spectral bands difficult to match. The curve which was determined for a solution prepared from the spleen of the first frog to be splenectomized was to the left of the normal curve on samples from the heart of frogs of similar size. For the last reason, solutions from the heart as well as from the spleen were used in subsequent experiments.

In this series of experiments thirteen frogs were used. One of these (Table I, no. 6) was not splenectomized, but was kept under the same conditions as the others as a control. No blank operations were performed.

The results from two frogs in which the solution from the spleen gave satisfactory curves are plotted on Fig. 1. These frogs appear in Table I as corresponding to intervals of 103 and 106 days. The specimens are thought to be typical, but analysis of Table I will show that the results from the spleen do not always have the same relation to those from the heart. In all cases where sufficient points on the curve could be accurately determined the curves are defined by the formula

\[ \frac{y}{100} = \frac{Kx^n}{1 + Kx^n}, \]
$y$ = percentage saturation, $x$ = partial pressure of oxygen. When only two points could be determined for solutions from the spleen the curve was plotted according to this formula.

To co-ordinate the data, logarithmic curves for each frog were plotted, and the oxygen pressure necessary to half-saturate the haemoglobin was determined. Since

\[
\frac{y}{100 - y} = Kx^n \quad \text{and} \quad \log \frac{y}{100 - y} = \log K + n \log x,
\]

these graphs are straight lines. The value of $n$ was found by determining the slope of the line—that is, the tangent of its angle with the abscissa. The constant $K$ can be determined by letting $y = 50$; then
The results are thus summarized in Table I. The value of $x$ when $y = 50$ is given rather than the value of $K$ because interpretation of the results is simplified.

**Table I.** Difference in the oxygen affinity of haemoglobin from the spleen and heart of a series of bullfrogs before and after splenectomy

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Body length mm.</th>
<th>Oxygen dissociation curves</th>
<th>Interval* days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
<td>Heart</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$pO_2$ for Hb=$HbO_2$ mm. Hg</td>
<td>$pO_2$ for Hb=$HbO_2$ mm. Hg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>$n$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial mm. Hg</td>
<td>Final mm. Hg</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>170</td>
<td>20.9</td>
<td>1.63</td>
</tr>
<tr>
<td>2</td>
<td>165</td>
<td>24.2</td>
<td>1.60</td>
</tr>
<tr>
<td>3</td>
<td>152</td>
<td>24.1</td>
<td>1.90</td>
</tr>
<tr>
<td>4</td>
<td>157</td>
<td>20.6</td>
<td>1.60</td>
</tr>
<tr>
<td>5</td>
<td>165</td>
<td>27.5</td>
<td>2.75</td>
</tr>
<tr>
<td>6</td>
<td>133</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>155</td>
<td>22.6</td>
<td>2.00</td>
</tr>
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<td>8</td>
<td>153</td>
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<tr>
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<tr>
<td>13</td>
<td>148</td>
<td>28.3</td>
<td>3.93</td>
</tr>
</tbody>
</table>

Interval after splenectomy between initial and final curves.

Examination of Table I shows that, with the exception of frogs 9 and 13, blood taken from the spleen had the same or a higher affinity for oxygen than that from the heart. Furthermore, in all splenectomized frogs, where a period of more than 25 days had elapsed after the operation, the curve shifted to the right; that is, the oxygen affinity decreased.

**DISCUSSION**

Variations in the position of curves of haemoglobin taken from the spleen might be expected if the spleen has any effect in a determination of the affinity for oxygen. There was no means for control of the amount of blood from the general circulation which was present in the spleen sample. Technical difficulties in determination of the curves from the spleen have already been discussed.

It does not seem profitable with the present knowledge of the subject to attempt to account for the higher affinity shown by the initial heart samples of specimens 9 and 10, or for other variations indicated by the results. In frog 10 the spleen curve was farthest to the left. But it will be noticed that the final curve for this frog is to the left of final curves for all other specimens. This indicates a consistency of results. The spleen curve of frog 9 was to the right of that for the heart, and the final curve approaches normal position for frogs of similar size. Size in individual cases is, of course, not necessarily a criterion of age or physiological development.
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The final readings for frog 5, one of the first to be splenectomized, were taken at the time the frog died without complete recovery from the operation. The specimen was very anaemic and otherwise abnormal; consequently, this curve is of doubtful value though the position corresponds with that of the initial curve on the spleen sample.

The outstanding feature of these results is the evidence that after splenectomy the oxygen dissociation curve of the haemoglobin shifts to the right. Previous study of frog blood has shown that there is a shift from left to right during the development of the bullfrog. This was considered to have an adaptive value in that the aquatic tadpole enjoys a haemoglobin of high affinity, whereas the air-breathing adult, which can forgo this high affinity, profits from a type with greater "unloading" capacity. The greatest shift to the right is in frogs measuring about 120-140 mm. body length. Further increase in length appears to be accompanied by a slight backward shift to a relatively uniform position. The suggestion that the bone marrow produces a type of haemoglobin different from that of the spleen or kidney seems to be supported by the results of this investigation.

At the beginning of this series of studies it was hoped that at least one of the specimens would have established a reversal of locus of erythrocyte formation to the primitive source, the kidney, as in tadpoles. However, examination of smears from all specimens showed active erythrocyte formation in the bone marrow.

In the production of erythrocytes—or possibly during the time erythrocytes are held in the spleen—this organ apparently influences the type of haemoglobin in that it increases the affinity of the pigment for oxygen. Knisely (1936) has shown in his studies of living rat and cat spleens that the erythrocytes are retained in the spleen, during what he calls the "storage phase", for periods varying from a few minutes to 10 hr. With reference to the name (storage phase) he says: "This term is used because the retention of blood cells is the visibly prominent feature. However, it is probable that the storage is accompanied by other, much more important processes."

That the spleen may be involved in the determination of the oxygen-carrying power of haemoglobin has been shown by Ray & Stimson (1927). They used splenectomized dogs and found that with reference to the blood the oxygen capacity decrease which follows the operation is more rapid than the decrease in total blood pigment. This indicates that there is produced a non-functional form of pigment. The work of Ray & Isaac (1930) with similar dogs gives evidence of the formation of a colourless form of haemoglobin ("leucohaemoglobin").

If the frog spleen and mammalian spleen are analogous organs, it seems quite possible from the results of this study that the spleen has a special function, namely, regulation of the oxygen affinity of haemoglobin. More extensive investigations of this possibility in mammals have been projected.
CONCLUSIONS

Removal of the spleen from frogs decreases the oxygen affinity of their haemoglobin. A corresponding increase in "unloading capacity" accompanies this change.

Haemoglobin in solutions obtained from frog spleens has an oxygen affinity which may be different from solutions prepared from blood in the general circulation. The oxygen affinity of the former is more often higher than that of the latter.

The results of this investigation indicate that the frog spleen as a locus of erythrocyte formation is a factor in the modification of the oxygen transport properties of blood. Possibly this is true because of an influence on the nature of haemoglobin without reference to the origin of the erythrocyte.

REFERENCES


