THE SIGNIFICANCE OF THE BOHR EFFECT IN
THE RESPIRATION AND ASPHYXIATION OF
THE SQUID, "Loligo Pealei"

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(With Three Text-figures.)

In 1904 Bohr, Hasselbalch and Krogh showed that carbon dioxide diminished the
affinity of haemoglobin for oxygen. This phenomenon, known as the “Bohr effect,”
together with the reciprocal effect of oxygenation upon the combination of carbon
dioxide with blood, discovered by Christiansen, Douglas and Haldane (1914) must
be taken into account in all quantitative considerations of the respiratory function
of the blood of mammals. It is now recognised that the effects of carbon dioxide
are not specific, but are due to alterations in the acid-base equilibrium of the blood
and may be produced by other acids. The phenomena have also been described in
the case of the blood of the lower orders of vertebrates (Krogh and Leitch, 1919;
and Southworth and Redfield, 1926) and of the body fluids of invertebrate animals,
such as the worms which have respiratory pigments more or less closely related to
haemoglobin (Barcroft and Barcroft, 1924).

Recently it was shown by Redfield and Hurd (1925) that blood containing
haemocyanin also exhibits the Bohr effect and that in the case of Limulus polyphemus
the action of carbon dioxide is reversed. Subsequent studies by Redfield, Coolidge
and Hurd (1926), Pantin and Hogben (1925), Hogben (1926), Hogben and Pinhey
(1926), Stedman and Stedman (1926) have extended these observations and lead
to several generalisations. The haemocyanins taken as a class vary in their affinity
for oxygen as the hydrogen-ion activity changes. The affinity for oxygen passes
through a minimum at some characteristic value of the hydrogen-ion activity. The
forms which have been studied fall into two groups. In one group the change in
affinity with hydrogen-ion activity is relatively great; the minimum affinity occurs
on the acid side of the physiological range, and as a result the production of carbon
dioxide in the respiratory cycle tends to increase the oxygen pressure of the blood
—the normal “Bohr effect.” This group includes the squid (Loligo), and the various
Crustacea (Homarus, Maia, Cancer). In the other group the minimum affinity

1 This phenomenon also occurs in the case of haemoglobin solutions as demonstrated by Rona
and Ylppö (1916) and Ferry and Green (1928).
occurs on the alkaline side of the physiological range and the Bohr effect consequently appears to be reversed when carbon dioxide is added to the blood. Moreover, in this group, which includes *Limulus, Busycon* and *Helix*, the affinity for oxygen is always relatively great and not much changed by alterations in the reaction of the blood.

In the present paper we will attempt to evaluate the importance of these phenomena to the respiratory physiology of the squid, *Loligo pealei*. This animal has been selected from among the members of the first group because in it the changes in oxygen affinity on adding CO₂ are very great, and because this organism is one of the most highly developed marine invertebrates when considered from the standpoint of activity.

The importance of the Bohr effect lies in the fact that the carbon dioxide produced in the tissues and eliminated in the respiratory organs alters the tension of oxygen in the blood in such a way as to facilitate the diffusion of oxygen from or to the blood. When it was noted how great an effect carbon dioxide has on the affinity of squid blood for oxygen (the blood is quite unsaturated when in equilibrium with human alveolar air) it was thought that this effect might be responsible for almost the entire respiratory exchange. Quantitative treatment requires an approximate description of the blood in respect to the simultaneous relations of total oxygen content, total CO₂ content, oxygen pressure, and CO₂ pressure, already available from the data of Redfield, Coolidge and Hurd (1926), together with knowledge of the change in composition of the blood in the course of the respiratory cycle. The latter has been determined by the following experiments.

Squids freshly taken from the trap were brought to the laboratory and at once pinned out on a board on the water table. A short ventro-lateral incision in the mantle exposed the heart. Two glass tubes, each bringing a vigorous jet of sea-water, were inserted under the anterior margin of the mantle on both sides and arranged so that fresh water was constantly washing the gills. The blood returning from the gills could now be seen to be dark blue. Samples of this blood were drawn in the following manner. A number 20 hypodermic needle was attached with rubber tubing close to the end of a pipette calibrated to deliver 1 c.c. between two marks. On inserting the needle through the posterior aorta into the cavity of the systemic ventricle the blood was forced at each beat into the pipette which became filled in about one-half minute. The sample was transferred at once to a Van Slyke blood-gas analyser, previously prepared, and analysed for CO₂ and O₂. A fresh animal was used for each sample.

From other animals venous blood, which appears almost colourless, was secured from the posterior sinuses and analysed. Table I contains the data obtained in this way. We may take 4·27 volumes per cent. oxygen and 3·98 volumes per cent. carbon dioxide as representative of the total gas content of arterial blood and 0·37 volumes per cent. oxygen and 8·27 volumes per cent. carbon dioxide as characteristic of the venous blood. These figures are concordant with those found by Winterstein (1909) in *Octopus vulgaris*. They yield a respiratory quotient of 1·1.

From the data of Redfield, Coolidge and Hurd (1926) the cartesian nomogram
represented in Fig. 1 has been constructed. For our purpose, it is an adequate
description of the squid’s blood at 23°C. The percentage saturation of haemocyanin
with oxygen at different O₂ and CO₂ pressures was taken from the curves in Fig. 3
of their paper. The oxygen capacity was taken to be 4.2 volumes per cent. The
absorption coefficient of oxygen in squid blood was taken as 0.021. The CO₂ dis-
sociation curve of reduced blood was taken from Fig. 5 of their paper. Oxygenation
is taken to reduce the CO₂ content of blood by 2 volumes per cent. at a CO₂ pressure
of 15 mm. Hg. It is assumed that at lower pressures the difference between the
CO₂ content of oxygenated and reduced blood is proportional to the CO₂ content
of reduced blood. The latter is the only assumption which it has been necessary to
make in constructing the diagram.

Table I.

<table>
<thead>
<tr>
<th>Arterial blood</th>
<th>Venous blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ volumes %</td>
<td>CO₂ volumes %</td>
</tr>
<tr>
<td>4.42</td>
<td>5.22</td>
</tr>
<tr>
<td>4.77</td>
<td>3.74</td>
</tr>
<tr>
<td>3.48</td>
<td>4.78</td>
</tr>
<tr>
<td>3.00</td>
<td>3.73</td>
</tr>
<tr>
<td>5.10</td>
<td>3.05</td>
</tr>
<tr>
<td>Mean 4.27</td>
<td>3.98</td>
</tr>
</tbody>
</table>

The arterial point, A, and the venous point, V, are marked on Fig. 1, and the
corresponding pressures of oxygen and carbon dioxide may be read off from the
non-rectangular co-ordinates. The line joining A and V is the locus of all possible
positions of the V point corresponding to the R.Q. of 1.1, and the A point as given.
Examination of the figure reveals the following interesting facts:

1. The blood is practically saturated with oxygen on leaving the gill.
2. The venous blood has given up nearly all its dissociable oxygen, although
the oxygen pressure has fallen only to 48 mm.
3. In taking up the corresponding amount of carbon dioxide the carbon dioxide
pressure has increased only 4 mm. It is clear that the gill is functioning with high
efficiency in oxygenating the blood. While considerable quantities of carbon dioxide
are retained in the blood, after passing the gill they correspond to a pressure of only
2 mm., so that equilibrium is nearly attained. The conclusion of Collip (1920) that
the body fluids of many invertebrates are not in equilibrium with sea-water as
regards carbon dioxide should be recalled.

The very great degree to which the oxygen of the blood is consumed in passing
through the tissues is striking and brings us to the question of the efficacy of carbon
dioxide in “turning out” oxygen from the blood at this point in the respiratory
cycle.

Fig. 1 indicates that in giving up 3.9 volumes per cent. oxygen to the tissues the
pressure of oxygen falls 70 mm. in the course of the respiratory cycle from A to V.
If carbon dioxide were not produced in the course of the cycle the change would be
Respiration of the Squid

from $A$ to $V_1$ along a line of constant CO$_2$ content. If we consider the maximum fall in O$_2$ pressure fixed at 70 mm., by the O$_2$ pressure obtaining at $V$, as the result of conditions within the tissue, the circulation rate, and the diffusion constant of oxygen, then this pressure would be reached at $V_1$ corresponding to an oxygen content of 1.9 volumes per cent. Under the circumstances only 2.3 volumes per cent. of oxygen would be given up. The difference between this value and 3.9 volumes per cent. of the normal cycle, or 1.6 volumes per cent., represents the oxygen exchange due to the influence of carbon dioxide on the system. Considered in terms of pressure, a fall in oxygen content corresponding to $A$—$V$, without attendant CO$_2$ production, would require a fall in pressure to the point $V_2$ corresponding to 23 mm. O$_2$ pressure. The production of CO$_2$ raises the O$_2$ pressure in the venous blood from that of $V_2$ to $V$, corresponding to about 25 mm. of oxygen pressure.

By similar reasoning it can be shown that the effect of reduction of the blood in the tissues increases the CO$_2$ content corresponding to the observed venous CO$_2$ pressure from 7.4 volumes per cent. (point $A_1$) to 8.3 volumes per cent. (point $V$). In terms of pressure, the CO$_2$ tension would rise to 8 mm. if the blood took up its normal load of CO$_2$ without simultaneous reduction, whereas the pressure actually rises to only 6 mm.

While the data on which these observations are based are statistical approximations, it may be concluded with confidence that the reciprocal effects of oxygen and carbon dioxide upon the respiratory properties of squid haemocyanin account for fully one-third of the respiratory exchange. The remaining two-thirds is attributable to differences in pressure between the gases of the tissues and of the sea-water bathing the gills.

It may be objected that the conditions under which the samples of blood were drawn were artificial and that the conclusions reached have little bearing on the normal respiratory physiology of the squid. There can be little question that the blood was adequately oxygenated and that the $A$ point consequently must be fairly correct. Under normal conditions the circulation may be sufficiently rapid to permit less complete utilisation of oxygen, in which case the $V$ point would move along the $A$—$V$ line toward $A$. Inasmuch as the co-ordinates are essentially symmetrical in this region of the nomogram, the relative importance of the factors determining the oxygen and carbon dioxide exchange would not alter greatly in such a case.

In collecting blood from the ventricle it appeared as though practically all the blood entered the needle rather than the other afferent vessels of the heart. That this should be so is not surprising when it is noted that rather high pressures exist in the arteries of Cephalapods—25 to 80 mm. Hg being recorded by Fuchs (1895) in the case of the Octopus. One may use the rate at which the pipette filled to gain an estimate at least minimal of the circulation rate in the squid. In a specimen weighing 180 gm. the pipette filled at a rate of 1 c.c. in 0.5 min. This yields a circulation rate of 2 c.c. per min., or 11 c.c. per kilo per min. Taking 3.9 volumes as the oxygen utilisation per 100 c.c. of blood it appears that 0.42 c.c. of oxygen are utilised per kilo per min. under the conditions obtaining when the blood was
drawn. Direct determination of the oxygen consumption of squids confined in aquaria of oxygenated sea-water yielded oxygen consumptions of about 10 c.c. per kilo per min. during the first 10 min. of their confinement. It seems improbable that when this very much larger oxygen requirement is met the utilisation of the oxygen per c.c. of blood is greatly diminished.

Fig. 1. Nomogram of the blood of the squid, *Loligo pealei*, illustrating the respiratory cycle. Temperature ca. 23°C. Rectangular co-ordinates denote total volume of oxygen and carbon dioxide in 100 volumes of blood. Sloping co-ordinates denote pressures of oxygen and carbon dioxide.

The change in the hydrogen potential of the blood in passing from the arterial to the venous state may be estimated from the data in Table I and Fig. 1. Taking 0.8 as the absorption coefficient of CO₂ in the blood, one may estimate from the CO₂ pressures the quantity of free CO₂, (H₂CO₃), in arterial and venous blood. Deducting this figure from the mean CO₂ content the CO₂ bound as bicarbonate, (BHCO₃), is obtained. Applying these results to the Henderson-Hasselbalch equation

$$pH = pK_1 + \log \left(\frac{BHCO_3}{H_2CO_3}\right)$$

the relative values of the pH of arterial and venous blood are secured. Knowledge of the exact value of $pK_1$ is unnecessary for the comparison. The results of this calculation are entered in Table II and indicate that the arterial blood is 0.13 pH units more alkaline than the venous blood. The value of this difference depends
Respiration of the Squid

The nomogram of the blood of the squid contains four sets of co-ordinates. Two of these, denoting oxygen and carbon dioxide content, describe conditions limited to the blood. Two others, the oxygen and carbon dioxide isopleths, describe factors common to the blood and its environment—at least under conditions when equilibrium between blood and sea-water may be assumed. The nomogram may in consequence be employed to describe those conditions of the environment, with respect to the respiratory gases, which are compatible with life, and in addition may aid us in interpreting the cause of death from asphyxiation. In particular it indicates the part played by oxygen and carbon dioxide pressure in determining the oxygen content of the arterial blood, and makes it possible to enquire to what extent the properties of the blood are responsible for death from asphyxiation.

We consequently caused squids to die of asphyxiation by placing them in closed vessels of sea-water saturated with air or variously enriched in carbon dioxide content. The composition of the sea-water at the time of death should give the environmental conditions just incompatible with life, and, since equilibrium between the blood and sea-water must be approached at this time, the nomogram may be employed to determine the corresponding oxygen and carbon dioxide contents of the blood.

Squids were obtained immediately after being brought from the nets, to insure their being in the best condition possible. Until the experiment they were kept in aquaria through which a full stream of sea-water was maintained. Aquaria containing from 5 to 8 litres of water were prepared, in many cases the sea-water being enriched in carbon dioxide by the addition of measured quantities of sea-water saturated with this gas. One squid was placed in each aquarium. The water was immediately covered with one-half inch of paraffin oil. A wire screen was placed beneath the layer of oil to prevent the squid from coming in contact with the oil and thus avoid any possible interference with the respiratory exchange through the introduction of oil into the mantle cavity.

After becoming progressively less active the animals underwent a series of
violent spasmodic swimming movements, then became quiescent. Respiratory movements ceased at this point. The tentacles twitched occasionally and continued to wave slowly for a short time. The chromatophores continued to pulsate for several hours. One hour after respiratory movements had ceased, on dissecting through the body wall, it was found that the hearts and the blood vessels of the gills were pulsating. Samples were taken for analysis 1 to 1½ hr. after the cessation of respiratory movement. It was believed that at this period decomposition had not set in, since the chromatophores and blood vessels were still contractile. With the cessation of respiratory movements, however, active exchange of gas between animal

![Graph](image)

Fig. 2. Pressures of oxygen and carbon dioxide (measured in mm. Hg) lethal to the squid. Solid circles represent lethal conditions in normal sea-water. Hollow circles represent lethal conditions in water initially enriched with carbon dioxide.

and sea-water must have nearly come to an end. Squids introduced into aerated sea-water ceased to respire in 40 to 60 min.; the addition of CO₂ to the water shortened the period from this figure down to 10 or 15 min., depending on the quantity added. The temperature of the water varied between 18° and 22° C. in the series of experiments.

Just prior to collecting the samples the water in the aquarium was gently stirred through the oil. Samples were siphoned into small bottles with ground stoppers, each sampling bottle being washed with two or three times its volume before the stopper was inserted. Upon one sample the oxygen content was determined by the Winkler method as described by Birge and Juday (1911). The corresponding oxygen pressures were estimated from the data of Fox (1909) for sea-water of salinity of
Respiration of the Squid

1800, and at the observed temperatures. The pH of another sample was determined colorimetrically, using phenol red or brom thymol blue as indicators. Salt error corrections of $-0.15$ for phenol red and $-0.19$ for brom thymol blue were employed (Saunders, 1924). The pressure of CO$_2$ in the sea-water was estimated from the pH values, using the data of Henderson and Cohn (1916).

The results of experiments performed in this way are shown in Fig. 2 by points plotted with reference to the estimated pressures of oxygen and CO$_2$ in the sea-water at death. Nineteen points, representing the lethal condition for animals placed in sea-water initially saturated with air, are grouped together in the region O$_2$ pressure = 20 to 35 mm.; CO$_2$ pressure = 0.8 to 1.4 mm. Points representing the lethal condition of animals placed in sea-water, to which an excess of CO$_2$ had been added, indicate that as the CO$_2$ pressure increases the squids die in the presence of higher and higher concentrations of oxygen. When these data are transferred to the nomogram of the blood (Fig. 3) an interesting result appears. Death occurs at those combinations of O$_2$ and CO$_2$ pressure which correspond to an O$_2$ content of 0.5 to 1.5 volumes per cent. It seems that in these animals death occurs when the environmental conditions are such that blood is unable to take up a sufficient quantity of oxygen from the gill.

Carbon dioxide might well be conceived to have a detrimental effect because of its acidic or other properties and independent of its action on the oxygen transport of the blood. If this were so one would expect squids to die in environments rich in CO$_2$ before the oxygen became reduced to a pressure incapable of saturating the blood sufficiently. At higher CO$_2$ pressures death should occur under conditions corresponding to higher oxygen contents and on the nomogram the lethal points should distribute themselves diagonally, shifting to the right as they moved upward. The absence of any such trend appears to negative this possibility.

Death seems to occur in animals exposed to sea-water enriched with CO$_2$ under conditions corresponding to rather lower oxygen content than is the case with animals asphyxiated in sea-water initially saturated with air. The difference is not great, but occurs consistently. It may be explained by the fact that in the presence of high CO$_2$ pressure the oxygen is held in the blood at high pressure. Its more complete utilisation by the tissues is thus facilitated.

The foregoing experiments afford proof that the respiratory pigment, haemocyanin, is vitally important to the squid. Life is impossible when conditions limit the oxygen content of the blood to as little as 0.5 volumes per cent. This is about the amount of oxygen dissolved in water or sera which contain no oxygen-binding protein when in equilibrium with the atmosphere. It is clear that such fluids could not transport sufficient oxygen to form a successful substitute for the blood of the squid.

Wells (1913) has observed that the asphyxial effects of carbon dioxide upon fish may be annulled by increasing the oxygen content of the water. Powers (1922) has conducted experiments very similar to ours upon fishes. They show that the ability of fish to utilise oxygen in asphyxia depends upon the hydrogen-ion activity of the sea-water. He explains these and other related data by an hypothesis
essentially similar to that which we have demonstrated to hold in the case of the squid. In view of the similarity of the Bohr effect in haemoglobin and in squid haemocyanin it appears probable that these facts could be quantitatively explained by a study of the physico-chemical properties of fish’s blood.

Fig. 3. Nomogram showing oxygen and carbon dioxide contents of the blood of the squid corresponding to environmental pressures of these gases measured in mm. Hg. Solid circles represent conditions lethal to squids placed in normal sea-water. Hollow circles represent conditions lethal to squids placed in sea-water previously enriched with CO₂. Rectangular co-ordinates denote total volume of oxygen and carbon dioxide in 100 volumes of blood. Sloping co-ordinates denote pressures of oxygen and carbon dioxide.

SUMMARY.

1. The oxygen and carbon-dioxide content of the arterial and venous blood of the squid, *Loligo pealei*, have been measured.

2. Using a nomographic method of analysis it is shown that the reciprocal effects of oxygen and carbon dioxide upon the respiratory properties of squid haemocyanin account for one-third of the respiratory exchange.

3. The venous blood is estimated to be 0.13 pH unit more acid than the arterial blood.
4. Death from asphyxiation occurs when the oxygen and carbon-dioxide pressures are such that the arterial blood can combine with only 0.5 to 1.5 volumes per cent. oxygen. Carbon dioxide exerts no toxic effect except through its influence on the oxygenation of the blood.

5. The haemocyanin of the blood is of vital necessity to the squid, because the amount of oxygen which can be physically dissolved in blood is less than the amount which is necessary for the maintenance of life.

**BIBLIOGRAPHY.**


