THE EFFECT OF LOW PRESSURES ON CELL OXIDATION

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INTRODUCTION

It has long been a matter of common knowledge that not only human beings but most other animals as well as plants can endure great variations in their supply of oxygen without obvious ill effects. This is demonstrated by the existence of life at high altitudes, the phenomena of anaerobiosis and many other facts. In addition to the fundamental researches of Harden, Hill, Meyerhof, Warburg, and many others, which have demonstrated the difference between, and yet the interdependence of aerobic and anaerobic respiration, numerous attempts have been made to correlate the amount of oxygen present with the degree of activity of an organism. One of the most recent of these is that of Amberson (1928) who shows quite definitely that in the case of Paramaecium and fertilized Arbacia eggs the cell respiration continues at the normal rate, almost independent of the oxygen supply until the latter reaches an exceedingly low value (about 10 mm. Hg). Warburg (1926) also noticed the same phenomenon in the case of yeast. Most other investigators’ results have agreed with these findings, among them those of Henze (1910), Lund (1918), Hamburger and St. Györgi (1925), Pütter (1924), Harvey (1925). The activities which were observed in this connection covered a wide range, including the actual uptake of oxygen itself (Amberson, Warburg). Mention may also be made of investigations concerning the effect of lowered oxygen tension on mammals (see Barcroft’s book, 1925). There seems to be little doubt, therefore, that within wide but varying limits the organism is independent of the oxygen supply.

The above considerations apply to changes in oxygen tension where the tension is varied by taking different proportions of oxygen and nitrogen but where the total pressure of the system is held constant.
at approximately 760 mm. Hg. Obviously the tension may also be varied by altering the total pressure where the relative gas tensions are held constant. This occurs both naturally and artificially when air is used and the barometric pressure is reduced (e.g., high altitudes or low pressure chambers). The classic work of Bert (1878) shows the diversity of effects which might be obtained by a lowering (or raising) of the barometric pressure. More recently interest in this connection has been centered on the influence of these conditions on human reactions, an interest which, of course, has been stimulated by the war and the increasing importance of aviation. Although the response of large animals to lowered pressures has been thoroughly studied very little attention has been paid to the effect of low pressures on the respiration of the individual cells and tissues themselves. The assumption has been tacitly made that the effect of a reduced oxygen tension whether secured by reducing the percentage of oxygen at atmospheric pressure, or by reducing the total pressure, would be the same. From the purely physical point of view such an attitude is very natural.

It seemed worth while, however, to test the validity of this assumption by a series of experiments in which the respiration of a simple organism would be measured under varying oxygen tension, these variations to be secured by altering both the percentage composition of the gases and the total pressure. The results of these experiments show quite clearly that there is a distinct and in some cases decided difference in the effect of gas tension depending on how the changes in tension are made. Or, in other words, it may be said that a reduced pressure, has, per se, an effect on tissue oxidation which is not obtained with a reduction of simply the oxygen tension.

**Methods**

The organism used in this work was baker's yeast, a fresh suspension of which was made up for each experiment in Ringer solution without bicarbonate, but containing 1 per cent glucose. The gas exchange was measured by means of a modification of the Warburg manometric method. The simple manometer, as well as the differential mano-

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1 J. Barcroft in his book, "The Respiratory Function of the Blood" (1925) and E. C. Schneider in a series of papers in the American Journal of Physiology have summarized what has been accomplished in this field.
meter, have been described fully in Warburg's book (1926), together
with details regarding the shaking device and temperature regulation.
The only modification of the method was made when it became neces-
sary to measure oxygen absorption or carbon dioxide evolution under
reduced barometric pressure.

For this purpose the simple manometer is clearly not adapted, since it is open
on one limb. It is also impossible to use the differential manometer in its usual
form because both limbs, or the containers attached thereto, must be initially at the
same pressure. Therefore a special differential manometer was constructed. The
manometer proper was of the usual type, very similar to illustration on page 1 of
Warburg's book ("Über den Stoffwechsel der Tumoren, 1926"), with posterior
extensions carrying two cups or vessels. Each of these was of the "Kegelgefäss"
or "conical vessel" type with a side extension and a small insert of glass on the
center of floor of the vessel for carrying alkali. Each of the side extensions was
fitted with a ground-in glass stopper. All joints were carefully ground and
greased and their resistance to leakage carefully checked. On the upward exten-
sion of each manometer limb there was a glass stopcock, A and B. Then the two
extensions were joined by fused glass tubing in the form of an inverted Y, at the
top of which was a three-way stopcock, C. Since the system was all of glass and
the only leaks could occur at the stopcocks. These were accordingly specially ground
in, greased, and tested frequently against leakage.

The fluid used in the manometer was Brodie's solution. In order to provide for
the introduction of the fluid it was necessary to open the bottom of the manometer
and fuse on a piece of tubing with another stopcock. When the latter was opened
the fluid could be run in to the desired level from below and the stopcock closed.
The manometer then functioned in the usual manner. The glass part was fastened
securely to a wooden back and carried on the shaker as described by Warburg. At
the beginning of an experiment the desired amount of yeast suspension was placed
in one of the vessels and an equal amount of Ringer solution in the other together
with any other material needed. The vessels were then placed on the manometer
and held in place by springs. If a special gas mixture was to be investigated, all the
upper stopcocks (A, B, and C) were opened and the small stoppers on the side
extensions of the vessels were removed. The gas was then allowed to flow through
the entire apparatus from a tank or other source of supply as described by Warburg
(1926, p. 3). Stopcock C was then closed and the small stoppers of the vessels
replaced. This isolated the system as a whole from the outside air. But the two
vessels remained in communication with each other through the arms of the in-
vverted Y. The vessels were then placed in the water bath at 37°C, and shaken till
temperature equilibrium had been attained. At this point stopcock C was opened
for an instant to relieve the positive pressure generated by the heating of the gas
inside the system. Then stopcocks A and B were closed. This cut off the com-
unication at the top between the two vessels and a differential alteration of pres-
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sure could then be observed on the manometer scale as in the case of any differential
manometer.

Suppose now after taking several readings it was desired to reduce the pressure
within the system. The stopcocks A and B were carefully opened thus equalizing
the pressure in the two vessels. A heavy rubber pressure tube was attached to the
outlet above stopcock C and a slight negative pressure put on. Cock C was then
opened and the pressure reduced to any predetermined amount. Then cock C was
closed and the same method used as under atmospheric pressure. If the pressure
was very low it was usually necessary to repeat this procedure two or three times
because after reduction the escape of gases from solution might alter the tension of
the gas space of the vessels.

To obtain reduced pressures an oil vacuum pump was attached to a 10 gallon
carboy. A line ran from the latter to the heavy tubing which could be attached
to the manometer. In this line was included a mercury manometer, scaled in
millimeters. Several heavy glass stopcocks variously placed allowed for cutting
off the line at any desired point. All joints were paraffined and shellacked. In
fact the system would hold a high vacuum without appreciable change for weeks.
The carboy was exhausted as highly as the pump would permit. This insured as
low pressures as were ever needed. After the stopcocks in front of and behind the
carboy had been closed the carboy served as a reservoir of low pressure to be drawn
on when desired.

For experiments with carbon monoxide it was necessary to generate the gas.
This was done by the method described in Treadwell's "Analytical Chemistry"
(11th German edition, 1923, vol. 2, p. 669), in which formic acid is run slowly into
hot concentrated H₂SO₄. The gas formed was run through a condenser and flasks
of water and soda lime, to remove water vapor and carbon dioxide. It was then
mixed in desired preparations with oxygen in a gasometer and thence run through
the manometer in the usual way.

The results may be expressed either on an absolute or relative basis. If the
latter, the normal respiration may be called 100 per cent and any deviation
expressed as a per cent of this normal. This method is often most convenient for
comparative data in which the absolute quantities involved are of secondary conse-
quence. However, for purposes of calculation the absolute amounts must be
known. In this case we may use as units the number of cubic millimeters oxy-
gen taken up or carbon dioxide evolved per milligram fresh weight of yeast per
hour. If we wished to compare different samples of yeast it would be necessary to
use the dry weight, but since in this work the same yeast was used throughout any
one experiment the fresh weight may be used. Then any two experiments may
be compared on a percentage basis.

Warburg (1926, pp. 8–10) has developed a formula for the calculation of volume
changes of gas in the differential manometer. He uses the following quantities:

\[ P = \text{Initial pressure in both vessels.} \]
\[ P_o = \text{The normal pressure (i.e., 760 mm. Hg) or 10,000 mm. Brodie.} \]
\( h = \) Observed difference of level in the manometer fluid.
\( h' = \) Increase in pressure in the compensation vessel.
\( \Delta \phi = \) Increase in pressure in the experimental vessel.
\( A = \) Cross section of the capillary.
\( V_G = \) Gas space in the experimental vessel.
\( V_G' = \) Same in compensation vessel.
\( V_F = \) Liquid space in the experimental vessel.
\( V_F' = \) Same in compensation vessel.
\( T = \) Absolute temperature.
\( \alpha = \) Absorption coefficient of the gas in the experimental vessel.
\( \alpha' = \) Same in compensation vessel.
\( x = \) Cm. \( \% \) of \( O_2 \) or \( CO_2 \) absorbed or evolved.

The increase of gas in the gas space of the experimental vessel is:

\[
\frac{P + \Delta \phi}{P_0} \frac{273}{T} \left( V_G + A \frac{h}{2} \right) - \frac{P}{P_0} \frac{273}{T} V_G. \tag{1}
\]

If the value of \( A \) is small then expression (1) becomes

\[
\Delta \phi \left( \frac{V_G}{P_0} \frac{273}{T} + A \frac{273}{T} \frac{P}{P_0} \right). \tag{2}
\]

He next assumes that \( P \) as a rule will equal \( P_0 \) and therefore \( \frac{P}{P_0} = 1 \). ("Hier ist \( A \frac{273}{T} \frac{P}{P_0} \) von der Größe eines Korrektionsgliedes. Bedenken wir, dass \( P \) in der Regel nahezu gleich \( P_0 \) ist, so können wir ohne merklichen Fehler den Faktor \( \frac{P}{P_0} \) gleich 1 setzen" . . . p. 9.) Therefore expression (2) reduces to:

\[
\Delta \phi \left( \frac{V_G}{P_0} \frac{273}{T} + A \frac{273}{T} \right). \tag{3}
\]

Furthermore the value of \( \Delta \phi \) is found to be

\[
h \left( 1 + \frac{A \frac{273}{T} \frac{P}{P_0}}{V_G' \frac{273}{T} V_F' \alpha'} \right). \tag{4}
\]

from which, in the numerator of the fraction within the parenthesis, the term \( P_0 \) has likewise been eliminated. In dealing with low pressures the assumption obviously does not hold that \( P \) approximates \( P_0 \). In fact it is usually widely differ-
ent. Hence the factor $\frac{P}{P_0}$ must be retained. The entire equation then is as follows:

$$x = h \left[ 1 + \frac{A \cdot \frac{273}{T} \cdot \frac{P}{P_0}}{V_G \frac{273}{T} + V_P \alpha} \left( \frac{V_G \frac{273}{T} + V_P}{P_0} + \frac{A \cdot \frac{273}{T} \cdot P}{P_0} \right) \right] \ldots \ldots (5)$$

The term enclosed by brackets in expression (5) is the so-called "Gefässkonstant" or "vessel constant" which, when multiplied by the observed change in the manometer fluid level, gives the amount of gas exchange in cubic millimeters. In this term the value $P$ occurs in the numerator twice. Therefore the magnitude of the term will vary directly as $P$. In other words, as the pressure in the system is lowered the vessel constant will decrease and vice versa. Thus if an organism absorbs the same number of molecules of oxygen per unit time as the pressure is lowered then the observed pressure change, $h$, must increase to compensate for the decrease in the vessel constant.

There are certain technical details which should be noted. The fluid in the manometer was Brodie's solution, used because the value of $P_0$ expressed in millimeters Brodie is an even 10,000. The volumes of the two sides of the manometer were determined by filling each side separately with mercury and weighing. The diameter of the capillary was similarly determined. The stopcocks were greased with a petroleum jelly-rubber composition which was firm enough to prevent leakage of air. Prior to actual use, the whole apparatus was set up empty in the water bath, exhausted as completely as possible, and shaken 2 hours. During this time no change in the fluid level of the manometer could be observed.

RESULTS

The Respiration and Fermentation of Yeast.—When the oxygen uptake of yeast is measured at different tensions the result is similar to that found by other investigators. There is little or no diminution in respiration until quite low tensions are reached. On the other hand, if the pressure is reduced the respiration falls off much more rapidly. This is illustrated by the data in Tables I and II.

In Tables I and II it is evident that the oxygen uptake decreases more rapidly when the pressure is reduced than when the pressure is maintained constant and the per cent of oxygen is cut down. A particularly clear instance of this occurs in Table II with a tension of 36 mm. of oxygen. In 5 per cent oxygen at normal pressure the
respiration is 84 per cent of that in air (144 mm.). When the pressure on a mixture containing 21 per cent oxygen, however, is reduced to 190 mm. giving the same partial pressure of oxygen (36 mm.) the respiration is cut down to 34 per cent of the normal. There is then clearly one effect on respiration which is brought about by a reduction of pressure, *per se*, and which is independent of the quantity of oxygen (or tensions).

**TABLE I**

3 cc. yeast suspension (10 mg. per cubic centimeter). Temperature 37° C. Initial gas mixture 100 per cent oxygen = 712 mm. oxygen tension. 2 0.4 cc. 10 per cent KOH in inset.

A

<table>
<thead>
<tr>
<th>Per cent O₂</th>
<th>Tension O₂ in mm. Hg</th>
<th>Respiration (mm. O₂ absorbed per mg. yeast per hour)</th>
<th>Per cent of normal (i.e., of respiration under standard conditions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>712</td>
<td>48</td>
<td>100</td>
</tr>
<tr>
<td>43</td>
<td>306</td>
<td>47</td>
<td>99</td>
</tr>
<tr>
<td>21</td>
<td>144</td>
<td>47</td>
<td>98</td>
</tr>
<tr>
<td>9</td>
<td>64</td>
<td>45</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>33</td>
<td>69</td>
</tr>
</tbody>
</table>

B

Per cent of oxygen maintained at 100 per cent, exclusive of water vapor, and barometric pressure varied.

<table>
<thead>
<tr>
<th>Barometric pressure in mm. Hg</th>
<th>760</th>
<th>600</th>
<th>500</th>
<th>400</th>
<th>300</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corresponding O₂ tension</td>
<td>712</td>
<td>552</td>
<td>452</td>
<td>352</td>
<td>252</td>
<td>152</td>
</tr>
<tr>
<td>Respiration (as above)</td>
<td>48</td>
<td>48</td>
<td>44</td>
<td>38</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td>Per cent of normal (as above)</td>
<td>100</td>
<td>100</td>
<td>92</td>
<td>79</td>
<td>75</td>
<td>33</td>
</tr>
</tbody>
</table>

Since respiration and fermentation (aerobic and anaerobic respiration) are closely related, experiments were performed to find out if the

2 It is of course necessary in all these experiments to subtract the vapor tension of the water present. At 37°C. this amounts to 48 mm. Hg at all barometric pressures. For example with 100 percent O₂ the total pressure is 760 mm. but the oxygen tension will be 760 – 48 = 712. If this same gas is reduced to a barometric pressure of, say, 300 mm. Hg then the oxygen tension will be 300 – 48 = 252. The same principle applies when the gas mixture contains any other percent of oxygen.
lowering of the pressure affected the latter as well as the former. This might be expected if the reduced pressure attacks the oxidation system of the cell as a whole. If, however, it is confined to that portion of the

### TABLE II

3 cc. yeast suspension (10 mg. per cubic centimeter). Temperature 37°C. Initial gas mixture 21 per cent oxygen, 79 per cent nitrogen (exclusive of water vapor). 0.4 cc. 10 per cent KOH in inset.

**A**
Average of two experiments, expressed as per cent of respiration with the initial mixture as the normal. Barometric pressure maintained at 760 mm. Mixtures as in Table I, A.

<table>
<thead>
<tr>
<th>Per cent O₂</th>
<th>21</th>
<th>9</th>
<th>5</th>
<th>1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tension O₂ in mm. Hg</td>
<td>144</td>
<td>64</td>
<td>36</td>
<td>11.5</td>
</tr>
<tr>
<td>Per cent of normal respiration</td>
<td>100</td>
<td>97</td>
<td>84</td>
<td>60</td>
</tr>
</tbody>
</table>

**B**
Average of five experiments, expressed in per cent of respiration in this mixture at 760 mm. Per cent of gases in the mixture, exclusive of water vapor: oxygen 21 per cent, nitrogen 79 per cent. Barometric pressure varied.

<table>
<thead>
<tr>
<th>Barometric pressure in mm. Hg</th>
<th>760</th>
<th>380</th>
<th>190</th>
<th>115</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corresponding O₂ tension</td>
<td>144</td>
<td>70</td>
<td>30</td>
<td>13</td>
<td>6.5</td>
</tr>
<tr>
<td>Per cent of normal respiration</td>
<td>100</td>
<td>74</td>
<td>34</td>
<td>27**</td>
<td>7**</td>
</tr>
</tbody>
</table>

* One experiment.
** Average of three experiments.

### TABLE III

3 cc. yeast suspension (10 mg. per cubic centimeter). Temperature 37°C. Initial gas nitrogen. Barometric pressure varied.

<table>
<thead>
<tr>
<th>Barometric pressure in mm. Hg</th>
<th>760</th>
<th>540</th>
<th>415</th>
<th>250</th>
<th>135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration: cmm. CO₂ produced per mg. per hour</td>
<td>45.5</td>
<td>46</td>
<td>45</td>
<td>45</td>
<td>46.5</td>
</tr>
<tr>
<td>Per cent of normal (i.e., per cent of respiration under standard conditions)</td>
<td>100</td>
<td>101</td>
<td>99</td>
<td>99</td>
<td>102</td>
</tr>
</tbody>
</table>

system which involves simply the uptake of oxygen then there might or might not be an effect on the anaerobic oxidations. Table III is an example of the results obtained. In this type of experiment the
manometer was filled with nitrogen gas which had been run through alkaline pyrogallol to remove all but the last traces of any oxygen that might be present. Such traces as remained would not affect the result materially. No KOH was placed in the vessel and the production of CO₂ was measured.

**TABLE IV**

3 cc. of 2 per cent fructose (Pfanstiehl) dissolved in N/2 Na₂CO₃. Temperature 37°C.

<table>
<thead>
<tr>
<th>Per cent of oxygen</th>
<th>100</th>
<th>21</th>
<th>9</th>
<th>5</th>
<th>1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen tension in mm. Hg</td>
<td>712</td>
<td>144</td>
<td>64</td>
<td>36</td>
<td>11.5</td>
</tr>
<tr>
<td>Cmm. O₂ per hour absorbed by fructose</td>
<td>530</td>
<td>329</td>
<td>180</td>
<td>81</td>
<td>12.5</td>
</tr>
<tr>
<td>Per cent absorption based on 100 per cent O₂ and 760 mm. as 100 per cent</td>
<td>100</td>
<td>62</td>
<td>33</td>
<td>15</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**A**

Initial gas 100 per cent O₂. Pressure maintained at 700 mm. Per cent of oxygen varied.

<table>
<thead>
<tr>
<th>Barometric pressure</th>
<th>760</th>
<th>345</th>
<th>195</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen tension in mm. Hg</td>
<td>712</td>
<td>297</td>
<td>147</td>
<td>32</td>
</tr>
<tr>
<td>Cmm. O₂ per hour absorbed by fructose</td>
<td>492</td>
<td>369</td>
<td>324</td>
<td>92</td>
</tr>
<tr>
<td>Per cent absorption based on 100 per cent O₂ and 760 mm. as 100 per cent</td>
<td>100</td>
<td>73</td>
<td>66</td>
<td>19</td>
</tr>
</tbody>
</table>

**B**

Initial gas 100 per cent O₂, exclusive of water vapor, maintained. Pressure varied.

<table>
<thead>
<tr>
<th>Per cent oxygen</th>
<th>9</th>
<th>20</th>
<th>43</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barometric pressure in mm. Hg</td>
<td>760</td>
<td>360</td>
<td>190</td>
<td>110</td>
</tr>
<tr>
<td>Oxygen tension in mm. Hg</td>
<td>63.5</td>
<td>63</td>
<td>63</td>
<td>63.5</td>
</tr>
<tr>
<td>Cmm. oxygen absorbed per hour</td>
<td>213</td>
<td>175</td>
<td>170</td>
<td>188</td>
</tr>
</tbody>
</table>

Both per cent of oxygen in nitrogen, exclusive of water vapor, and the barometric pressure were simultaneously varied so as to bring the oxygen tension in all cases to 63 to 64 mm. Hg. The variation in the tension was not over 1 per cent.

This experiment, which was checked several times, shows that there is no such diminution of anaerobic respiration with reduced pressure as is experienced with aerobic respiration. In fact there may be said to be no effect at all. The significance of this result is discussed below.

**Artificial Systems.**—The question arises: Is the different action of
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reduced oxygen tension and reduced pressure due to a purely physical cause having to do with the solution and diffusion of the gas, or is it dependent upon the organization of the cell itself? In this connection the usual type of experiment was performed with two artificial oxidation systems which proceed in homogeneous solution: the absorption of oxygen by fructose in alkaline solutions and the oxidation of cysteine. These systems have been studied exhaustively by Warburg and his co-workers who have shown that both are oxidations catalyzed by metals.³ (Sakuma, 1923; Warburg and Yabusoe, 1924; Krebs, 1927).

The results are indicated in the data cited in Tables IV and V.

The results shown in Table IV indicate that, apart from experimental error, the amount of oxygen uptake with fructose is dependent on the oxygen tension alone and is not affected materially by changes of pressure. In other words the initial concentration of oxygen is the determining factor.

The typical experiment cited in Table V indicates that the cysteine system behaves analogously to the fructose system in that the oxidation is dependent solely on the oxygen tension.

**The Inhibition of Respiration by Carbon Monoxide.**—The long series of well known facts concerning the action of carbon monoxide on hemoglobin and its capacity to inhibit tissue oxidation (Warburg, 1926) lends support to the idea that we have, in carbon monoxide, a substance which behaves in a manner very similar to oxygen. It

³ No metals were added to these systems because, although fructose and cysteine of the highest obtainable commercial purity (Pfanstiehl) were used and all possible precautions observed, there were sufficient impurities in the form of metals (chiefly iron) to catalyze the oxidations.
may be said to compete with oxygen in the series of reactions which we call oxidation. It seemed therefore advisable to see if low pressure would prevent the uptake of carbon monoxide as well as that of oxygen. This could, of course, be measured by the inhibition of the oxygen uptake. For just so much as the carbon monoxide replaces the oxygen in the oxidation system, by just so much is the normal absorption of oxygen diminished.

The work of Warburg (1926) indicated that when the proportion of carbon monoxide to oxygen is increased the toxicity of the former increases. Since in this investigation it was intended to reduce the pressure on a constant mixture of carbon monoxide and oxygen and thereby decrease the total quantity of both gases, it was of importance to know definitely, apart from the question of pressure, whether the effect of carbon monoxide on oxidation could be varied by keeping the ratio $\frac{CO}{O_2}$ constant and varying the total amounts, or whether, as is implied by Warburg's work, it is solely a matter of the ratio $\frac{CO}{O_2}$.

To be certain of the situation two series of experiments were performed: first a series where the ratio of carbon monoxide to oxygen was kept constant but the total quantity of both was altered at 760 mm., and secondly a series where the ratio was altered. In both these series the respiration at a given oxygen tension with carbon monoxide had to be compared with the respiration at the same oxygen tension where nitrogen replaced the carbon monoxide. This, of course, was to control the normal moderate reduction of respiration due to the diminished oxygen tension itself.

In the first series (Table VI) an initial mixture of 21 per cent $O_2$ and 79 per cent $N_2$ was used to determine the normal respiration. Then a mixture of 21 per cent $O_2$, 79 per cent CO was substituted. The latter was then diluted with nitrogen to reduce the total concentration of both oxygen and carbon monoxide to the same degree.

In the second series (Table VII) the ratio $\frac{CO}{O_2}$ was changed by filling the manometer successively with different mixtures of the two gases.

Here it will be seen that replacing 79 per cent nitrogen with an equivalent amount of carbon monoxide reduced the oxygen uptake
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TABLE VI

<table>
<thead>
<tr>
<th>Gas mixture</th>
<th>21% O₂</th>
<th>21% O₂</th>
<th>10% O₂</th>
<th>5% O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>79% N₂</td>
<td>79% CO</td>
<td>40% CO</td>
<td>20% CO</td>
</tr>
<tr>
<td>Oxygen tension in mm. Hg.</td>
<td>144</td>
<td>144</td>
<td>72</td>
<td>36</td>
</tr>
<tr>
<td>Per cent respiration on basis of 21 per cent O₂ in N₂ as 100 per cent</td>
<td>100</td>
<td>54</td>
<td>50</td>
<td>36</td>
</tr>
</tbody>
</table>

TABLE VII

Comparison of mixtures of oxygen with nitrogen and carbon monoxide. Temperature 37°C. Pressure maintained at 760 mm. Hg. 3 cc. yeast, 10 mg. per cubic centimeter. 0.4 cc. 10 per cent KOH in inset.

<table>
<thead>
<tr>
<th>Gas mixture</th>
<th>43% O₂</th>
<th>21% O₂</th>
<th>9% O₂</th>
<th>5% O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>57% N₂</td>
<td>79% N₂</td>
<td>91% N₂</td>
<td>95% N₂</td>
</tr>
<tr>
<td>Oxygen tension in mm. Hg.</td>
<td>306</td>
<td>144</td>
<td>64</td>
<td>36</td>
</tr>
<tr>
<td>Respiration in cmm. oxygen absorbed per hour per mg. yeast</td>
<td>39.7</td>
<td>39.0</td>
<td>37.3</td>
<td>27.5</td>
</tr>
<tr>
<td>Per cent respiration on basis of 43 per cent O₂ 57 per cent N₂ as 100 per cent</td>
<td>100</td>
<td>98</td>
<td>94</td>
<td>69</td>
</tr>
</tbody>
</table>

* Per cent inhibition is represented by the expression:

\[
\frac{100 \left(\text{per cent respiration in N₂} - \text{per cent respiration in CO}\right)}{\text{per cent respiration in N₂}}
\]

to about 54 per cent. Further reduction of the concentration of both gases in equal degree at constant pressure causes an added reduction. But this may be accounted for on the basis of reduction of oxygen.
tension alone. If we call the oxidation in the presence of 21 per cent O₂, 79 per cent CO equal to 100 per cent then with 10 per cent O₂, 40 per cent CO it is 92.5 per cent and with 5 per cent O₂, 20 per cent CO it is 67 per cent. This is slightly greater than, but of the same order of magnitude as, what would be expected on the basis of the oxygen-nitrogen mixtures previously investigated (Table I). Therefore it may be said that if the ratio remains constant the inhibition remains sensibly the same.

What happens when the ratio is altered may be seen in Table VII where it is very evident that the inhibitory effect of carbon monoxide increases as the ratio \( \frac{CO}{O_2} \) increases and vice versa. When the pressure is reduced on a constant mixture of two such gases of low solubility their ratio in solution will remain practically constant. If the reduced pressure has an equal effect on the reactions of the cell itself with respect to the two gases the degree of inhibition on the part of the carbon monoxide will not be altered. If on the other hand, the degree of inhibition is altered it may be taken as an indication that low pressures affect the reactions of the two gases differentially in the cell.

We may now proceed to what happens when the pressure is reduced.
The data covering this situation are summarized in Table VIII. In all experiments two parallel series were run, first with 21 per cent O₂, 79 per cent N₂, then, on the same yeast with 21 per cent O₂, 79 per cent CO. The inhibition due to the carbon monoxide was then calculated.

From Table VIII one significant conclusion may be drawn. When the pressure is reduced the inhibition progressively disappears until it may be said to be entirely removed. In others words, low pressures have a greater effect on carbon monoxide than on oxygen with respect to their relative power to affect the oxidation system of the cell.

**DISCUSSION**

There are certain theoretical considerations which may be discussed with regard to the foregoing experimental evidence without attempting to build up any complete hypothesis to account for the effect of low pressures on cell oxidations.

1. That living oxidation systems are affected by low pressures to the exclusion of artificial systems has been shown by the fact that the respiration of yeast is reduced by low pressures over and above what can be accounted for by simple decrease in oxygen concentration (tension) whereas such low pressures seem to have no extra effect whatever on artificial systems of the fructose or cysteine type. The rate of the latter is governed solely by the number of oxygen molecules present. This proves nothing but what is already universally admitted, that in the complex and heterogeneous oxidation system of the cell there is a mechanism which cannot be duplicated in all respects by a relatively simple, homogeneous, system in vitro. We may however, narrow down the field to some extent, for any purely concentration effects due to the action of reduced pressure on a homogeneous system may be ruled out.

2. The lack of any influence of low pressures on the anaerobic respiration of the yeast indicates that not all of the oxidation system of the cell is affected, but only that part which has to do with the uptake of oxygen. We must assume the existence of some kind of catalyst in the cell which facilitates this uptake either by direct union with oxygen, or by causing it to combine, temporarily at least, with some substance from which the oxygen can be carried to the final
oxidisable materials in the cell. The possibility of an organic peroxide immediately suggests itself, or a haem derivative on the order of hemoglobin or Warburg’s “Atmungsferment.” It is not necessary to postulate any definite chemical entity but is sufficient to assume merely the presence of some substance with which the oxygen combines.

It is known that some peroxides such as hydrogen peroxide are relatively stable in contact with inert gases like nitrogen at normal pressure, but are very easily decomposed if the pressure is lowered. The equilibrium between hemoglobin and the oxygen tension has likewise been elucidated although in this instance it is generally understood that the equilibrium is the same regardless how the partial pressure of the oxygen is achieved (whether by gas mixtures or by reduction of the barometric pressure). At all events the suggestion may be made that one of the intermediate compounds of oxygen in the cell is of such a nature that it is broken up to a greater or less extent by lowering the barometric pressure. Conversely, of course, a low pressure would prevent the formation of some of this compound in the first place and would thus decrease the amount of oxygen which could eventually be used by the cell.

3. The data secured with carbon monoxide are of interest in this connection. Since it has been shown quite definitely that the degree of inhibition on the part of carbon monoxide depends upon the ratio \( \frac{CO}{O_2} \) itself, at the point where oxidation is taking place, the ratio must be altered by low pressure. If we make the further assumption that the intermediate compound previously mentioned may be formed with carbon monoxide as well as oxygen then we have only to say that low pressures decompose the compound with carbon monoxide more readily than that with oxygen. Warburg and Negelein (1928) have proved quite conclusively that there exists a CO- “Atmungsferment” compound which is decomposed by light. This is itself evidence of a relatively unstable compound. It seems also reasonable to suppose that this, or a similar, substance may be decomposed by low pressure.
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SUMMARY

1. A method is described for measuring tissue oxidation under reduced barometric pressure.
2. The oxygen uptake of yeast is diminished by low barometric pressures to a greater extent than by a reduction of the partial pressure of oxygen, to a corresponding degree, at atmospheric pressure.
3. This effect of low pressure is not observed with certain in vitro oxidation systems.
4. The anaerobic respiration (carbon dioxide production) of yeast is not at all affected by low pressures.
5. The inhibition of tissue oxidation caused by carbon monoxide is removed by lowering the pressure.

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BIBLIOGRAPHY

Bert, P., La Pression Barometrique, Paris, 1878.
Harvey, E. N., J. Gen. Physiol., 1925, 8, 89.
Henzel, M., Biochem. Z., 1910, 26, 225.
Pütter, A., Arch. ges. Physiol., 1924, 204, 94.
Sakuma, S., Biochem. Z., 1923, 142, 68.
Warburg, O., Biochem. Z., 1926, 177, 471.
Warburg, O., and Yabusee, M., Biochem. Z., 1924, 146, 380.