THE RELATION OF OXYGEN SUPPLY TO WATER MOVEMENT
AND TO UREA FORMATION IN SURVIVING
LIVER TISSUE

BY EUGENE L. OPIE

(From The Rockefeller Institute)

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In a preceding publication (1) evidence was presented to show that surviving liver cells in the presence of oxygen produce urea and lose water, whereas with anoxia little, if any, urea is formed and water enters the cells in considerable quantity. It seemed probable that intracellular amino acids and substances derived from them—for example, arginine, ornithine and citrulline—can maintain a high intracellular osmotic pressure but with formation of urea, leave the cells. Kidney cells which form little, if any, urea undergo swelling both in the presence and absence of oxygen.

The purpose of the present study has been to determine how a varied supply of oxygen influences the movement of water in surviving liver tissue. The attempt has been made to imitate in vitro as closely as possible conditions present during life.

Slices of liver tissue have been immersed in Krebs-Ringer solution with all electrolytes 0.154 molar and with bicarbonate buffer. The method which has been used has been described in detail in preceding publications (1, 2). Liver slices preferably weighing from 50 to 100 mg. have been immersed in 25 cc. of the medium in flasks of Erlenmeyer type in a water bath at 38°C. and with rhythmic shaking at the rate of about 130 per minute. Gas mixtures have streamed through the shaking flasks. In all instances the gas mixtures have contained 5 per cent of carbon dioxide. Anoxia has been produced by nitrogen 95 per cent with carbon dioxide 5 per cent. Oxygen has been used varying from 1 to 95 per cent with carbon dioxide 5 per cent and nitrogen in the remaining volume.

Ehrlich (3) first demonstrated the scant oxygen content of the extravascular tissues. When alizarin blue in saturated solution was injected into the blood stream of rabbits, tissues were found to be unstained when the body was opened, but within 10 minutes of exposure to the air, tissues, including liver and lungs, became blue. Alizarin blue had been reduced within the tissues to form a colorless compound which with exposure to the air took the usual blue color. For a time, on the basis of this experiment it was assumed that the extravascular tissue contained no free oxygen but later investigation modified this opinion.

When Campbell (4) injected a liter of nitrogen into the subcutaneous tissue of the back of rabbits the gas was absorbed slowly; more than half remained after a week and some was not absorbed after 3 to 5 weeks. Oxygen tension within the gas mixture
very soon measured 40 to 50 mm. Hg and after 1½ to 3 days remained constant be-
tween 20 and 30 mm. Hg. Similar changes were observed when nitrogen was injected
into the peritoneal cavity. Carbon dioxide tension in pockets of nitrogen within the
subcutaneous tissue or peritoneal cavity measured almost uniformly 40 to 50 mm.
Hg. Pure oxygen disappeared within about 1 week and carbon dioxide after an hour.
When air was injected, nitrogen was absorbed slowly. In a later publication Campbell
(5) described the same changes in rats, guinea pigs, and cats. Oxygen tension in the
subcutaneous tissue and peritoneal cavity of monkeys was somewhat higher (40 mm.
Hg) than in the other animals tested.

Water Movement in Liver Slices with Varied Oxygen Supply

Figures measuring approximate gas tensions in alveolar air, arterial blood,
and venous blood (6), widely accepted with slight modifications, are presented
in Table I.

<table>
<thead>
<tr>
<th></th>
<th>Air</th>
<th>Alveolar air</th>
<th>Arterial blood</th>
<th>Venous blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxygen</strong></td>
<td>158.0</td>
<td>100</td>
<td>95</td>
<td>40</td>
</tr>
<tr>
<td><strong>Carbon dioxide</strong></td>
<td>0.3</td>
<td>40</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td><strong>Water vapor</strong></td>
<td>5.7</td>
<td>47</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td><strong>Nitrogen</strong></td>
<td>596.0</td>
<td>573</td>
<td>573</td>
<td>573</td>
</tr>
</tbody>
</table>

In the present experiments with streaming gas over a shaking fluid surface
water vapor forms part of the mixture surrounding slices of liver tissue. The
partial gas tensions of the mixtures that have been used have been presented
in Table II.

All of these mixtures (Table II) contain carbon dioxide 5 per cent with a
partial pressure close to that in alveolar air and in arterial blood, namely, 40
mm. Hg. The oxygen tension in the subcutaneous tissue and in the peritoneal
cavity of the rabbit as indicated by the experiments of Campbell may vary
from 20 to 30 mm. Hg and is approximately 3.5 per cent. It has been assumed
that 95 per cent of air (about 150 mm. Hg) in the present experiments supplies
oxygen to the immersed liver slices in quantity somewhat greater than that
brought by the hemoglobin of the red blood corpuscles to the tissues. When
oxygen pressure is 150 mm. Hg, hemoglobin is saturated with oxygen whatever,
within physiological limits, the pressure of carbon dioxide (6); but at lower
oxygen pressure diminution of carbon dioxide pressure may increase the oxygen
held by hemoglobin.
Movement of water in liver slices presumably anoxic when immersed in Krebs-Ringer solution (Table I, Fig. 1), gassed with a mixture of 95 per cent of nitrogen and 5 per cent carbon dioxide, has been compared with that in slices exposed to a gas mixture of air and 5 per cent of carbon dioxide, with partial oxygen pressure of 150 mm. Hg. Similarly, the effect of oxygen tension increased to 95 per cent has been determined although it is evident that this concentration is much greater than that possible under physiological conditions. Table III and Fig. 1 show the average change of weight of liver slices during 10, 60, 120, 180, and 240 minutes.

In the presence of the three gas mixtures water has entered the tissue rapidly during the first 10 minutes of immersion. With anoxia water intake has continued and reached a maximum after 180 minutes; later, water has been lost.

### TABLE II

*Approximate Partial Pressure in Gas Mixtures*

<table>
<thead>
<tr>
<th>Gas Mixture</th>
<th>Oxygen</th>
<th>Carbon dioxide</th>
<th>Nitrogen</th>
<th>Water vapor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen 95 per cent</td>
<td>0</td>
<td>36</td>
<td>677</td>
<td>47</td>
</tr>
<tr>
<td>Oxygen 1 per cent</td>
<td>7</td>
<td>36</td>
<td>670</td>
<td>47</td>
</tr>
<tr>
<td>Oxygen 3.5 per cent</td>
<td>25</td>
<td>36</td>
<td>652</td>
<td>47</td>
</tr>
<tr>
<td>Oxygen 10 per cent</td>
<td>71</td>
<td>36</td>
<td>606</td>
<td>47</td>
</tr>
<tr>
<td>Oxygen 14 per cent</td>
<td>100</td>
<td>36</td>
<td>578</td>
<td>47</td>
</tr>
<tr>
<td>Oxygen 20 per cent</td>
<td>143</td>
<td>36</td>
<td>535</td>
<td>47</td>
</tr>
<tr>
<td>Air 95 per cent</td>
<td>150</td>
<td>36</td>
<td>528</td>
<td>47</td>
</tr>
<tr>
<td>Oxygen 25 per cent</td>
<td>178</td>
<td>36</td>
<td>499</td>
<td>47</td>
</tr>
<tr>
<td>Oxygen 95 per cent</td>
<td>677</td>
<td>36</td>
<td>0</td>
<td>47</td>
</tr>
</tbody>
</table>

In the presence of air, 95 per cent, water present after 10 minutes has diminished rapidly during the remainder of the 1st hour and still more during the period from 2 to 4 hours. When gassed with 95 per cent oxygen water movement in the liver slices has followed a course similar to that in air but at a much lower level.

With the progress of these experiments it became evident that the movement of water in immersed slices of liver varied widely when the liver tissue was obtained from animals at different periods following the ingestion of food and was in part determined by the stage of gastrointestinal digestion at the time when the liver tissue was obtained. At an early period of digestion when the stomach was distended with food, immersed liver slices took up much more water than those obtained at a later period of digestion when the stomach content was decreasing and intestinal digestion was well under way. This relation will be described in a later publication.

If the factor of gastrointestinal digestion were ignored average figures might
Fig. 1. Movement of water in immersed liver slices exposed to varied oxygen supply. See Table III.

give uncertain information about the relation of water movement in liver slices to the oxygen supply available to them. To eliminate variations in the conditions affecting different animals liver slices from one animal have been exposed to gas mixtures with different partial pressures of oxygen. With the procedure that has been used it has been necessary to limit to six, tests made with liver slices from one animal. In some experiments water movement has
been measured after exposure to oxygen in three concentrations during 120 and 180 minutes of immersion (Fig. 2, Experiments 1 and 6) and in others to two concentrations during 60, 120, and 180 minutes (Fig. 2, Experiments 2, 3, 4, 5, 7, and 8). Inspection of the graphs shows that water intake after 60 minutes in most instances is approximately the same in the presence of different concentrations of oxygen and differences in water content of slices dependent upon oxygen supply are best measured after 120 and 180 minutes.

The addition of 1 per cent of oxygen to an otherwise anoxic gas mixture has caused no significant change of water intake (Fig. 2, Experiments 3, 4, and 5). With oxygen 3.5 per cent water content of liver tissue after 120 or 180 minutes of immersion has been less than that with nitrogen (Fig. 2, Experiment 1) and greater than that with oxygen 10 per cent (Fig. 2, Experiments 6 and 8) or

| TABLE III |
| Water Movement in Liver Slices Exposed to Gas Mixtures with Varied Oxygen Content |

Gas mixtures are given in the table in accord with their per cent of oxygen content. Each contains 5 per cent of carbon dioxide and nitrogen in all instances up to 100 per cent. Figures in parenthesis in this and in other tables indicate the number of determinations.

<table>
<thead>
<tr>
<th>Water—Per Cent of initial weight</th>
<th>After 10 min.</th>
<th>After 60 min.</th>
<th>After 120 min.</th>
<th>After 180 min.</th>
<th>After 240 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen 95 per cent . . . . . . .</td>
<td>106.8 (3)</td>
<td>101.3 (8)</td>
<td>97.7 (10)</td>
<td>96.1 (6)</td>
<td>84.2 (12)</td>
</tr>
<tr>
<td>Air 95 per cent . . . . . . . .</td>
<td>112.6 (1)</td>
<td>106.3 (5)</td>
<td>105.6 (13)</td>
<td>103.3 (10)</td>
<td>97.9 (4)</td>
</tr>
<tr>
<td>Nitrogen 95 per cent . . . . .</td>
<td>109.3 (2)</td>
<td>116.1 (6)</td>
<td>120.5 (10)</td>
<td>117.3 (4)</td>
<td>111.3 (11)</td>
</tr>
</tbody>
</table>

with air (Fig. 2, Experiments 1 and Fig. 3, Experiment 11). In Experiment 6 of Fig. 2 the relation of water content of tissue in the presence of oxygen 20 per cent to that with oxygen 3.5 and to 10 per cent has not been in accord with other experiments and may be the result of inadvertent error.

The graphs of Fig. 3 depict water movement in liver slices exposed to 15 per cent oxygen (about 100 mm. Hg), 20 per cent (about 143 mm. Hg) and to air (about 150 mm. Hg) under the conditions that have been described. The partial oxygen pressure of arterial blood (Table I) is approximately 95 to 100 mm. Hg and this is presumably the highest oxygen tension to which the tissues can be exposed during life. Water movement in the presence of these gas mixtures has shown no constant variation corresponding with their oxygen concentration. In Experiment 9 liver slices with oxygen 25 per cent have lost more water than those exposed to air but in Experiments 10 and 11 the reverse is true. In Experiment 12 water content of liver slices in the presence of oxygen 20 per cent has been less than that of slices in contact with air. In Experiment 13, as might be expected, water movement has been almost the same with oxygen 20 per cent
Fig. 2. Experiments showing water movement in immersed liver slices in each instance from the same animal and with varied oxygen supply. O is oxygen; A, air; N, nitrogen. Here and elsewhere numbers have been assigned to experiments with no reference to the order in which they were made.
and with air 21 per cent oxygen. It can be noted (Experiment 12) that water movement with 15 and with 20 per cent oxygen have differed little.

Experiments (Table IV, Experiments 15 to 18) were made to compare movement of water in liver slices exposed to oxygen 14 per cent (about 100 mm. Hg, Table II) and that in the presence of air (about 150 mm. Hg, Table II). In
each of four experiments liver slices with one or other gas mixture were from the same animal, which had received no food during 20 hours. Water movement under the comparable conditions was the same, and suggest that within a range of oxygen supply, maximum under physiological conditions, minor variations have scant influence upon the movement of water in the liver slices.

In Fig. 4 are graphs showing water movement in liver slices in the presence of gas mixtures with oxygen concentration varying widely from 25 to 95 per cent (Experiments 15 to 21). In all instances water content of liver slices has decreased in accord with the increasing concentration of oxygen but the extent of the deviation has evidently varied with other factors. It is evident that these concentrations of oxygen cannot occur in the tissues during life.

### TABLE IV

*Water Movement in Liver Slices Exposed to Oxygen 14 per cent and Air 95 per cent*

<table>
<thead>
<tr>
<th></th>
<th>With oxygen 14 per cent</th>
<th>With air 95 per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 60 min.</td>
<td>After 120 min.</td>
</tr>
<tr>
<td>Experiment 15</td>
<td>132.6</td>
<td>128.4</td>
</tr>
<tr>
<td>&quot;</td>
<td>128.4</td>
<td>125.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>130.2</td>
<td>129.1</td>
</tr>
<tr>
<td>&quot;</td>
<td>127.0</td>
<td>132.8</td>
</tr>
<tr>
<td>Average</td>
<td>129.6</td>
<td>128.0</td>
</tr>
</tbody>
</table>

### Urea Formation with Varied Oxygen Supply

As already stated liver slices form urea in the presence of oxygen, but with anoxia little, if any, is formed. A preceding publication (1) presented evidence that the intracellular osmotic pressure of liver cells removed from the body is referable in part to amino acids and related substances which with oxidation leave the cells as urea. In mammalian species the nitrogen of protein through widely varied stages leaves the body almost wholly as urea. The nitrogen of amino acids that have been introduced into the blood stream has this fate. The experiments of Bollman, Mann, and Magath (7) show that the liver, with perhaps insignificant exceptions, is the only site of formation of urea.

The relation of oxygen supply to urea formation by liver slices under the conditions of these experiments has been studied. The gas mixtures used have contained oxygen 95, 25, and 3.5 per cent, air 95 per cent and nitrogen 95 per cent, in all instances with 5 per cent of carbon dioxide and when necessary nitrogen in quantity up to a total of 100 per cent. Table V and Fig. 5 record urea formation in mg. per 100 gm. of tissue before immersion and after 10, 60, 120, 180, and 240 minutes. The average urea content of liver slices after
Fig. 4. Movement of water in immersed liver slices as in Fig. 2.

removal from the body has been from 30.6 to 39.4 and in some instances has varied from 21 to 67 mg. During the first 10 minutes of immersion there has been a fairly uniform increase of urea coincident with the increase of water content of the tissue. Urea formation save in the first 10 minutes has later in-
TABLE V
Formation of Urea by Liver Slices Exposed to Gas Mixtures with Varied Oxygen Content

<table>
<thead>
<tr>
<th></th>
<th>Immediate</th>
<th>After 10 min.</th>
<th>After 60 min.</th>
<th>After 120 min.</th>
<th>After 180 min.</th>
<th>After 240 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen 95 per cent</td>
<td>39.4 (10)</td>
<td>46.7 (3)</td>
<td>84.3 (7)</td>
<td>78.1 (5)</td>
<td>101.6 (3)</td>
<td>116.9 (8)</td>
</tr>
<tr>
<td>Oxygen 25 per cent</td>
<td>32.2 (5)</td>
<td>44.3 (2)</td>
<td>42.6 (2)</td>
<td>62.7 (4)</td>
<td>91.4 (2)</td>
<td>82.8 (3)</td>
</tr>
<tr>
<td>Air 95 per cent</td>
<td>30.6 (6)</td>
<td>62.9 (1)</td>
<td>39.9 (1)</td>
<td>57.9 (5)</td>
<td>86.6 (2)</td>
<td>75.7 (4)</td>
</tr>
<tr>
<td>Nitrogen 95 per cent</td>
<td>30.9 (12)</td>
<td>40.3 (2)</td>
<td>34.0</td>
<td>41.6 (9)</td>
<td>42.5 (3)</td>
<td>38.9 (11)</td>
</tr>
</tbody>
</table>

Fig. 5. Urea formation by immersed liver slices with varied oxygen supply. See Table V.

creased in all instances in the presence of increasing oxygen but has failed to occur with nitrogen.

Amino Acid Formation with Varied Oxygen Supply
Evidence that the liver is the site of deamination of amino acids and of the formation of urea is based in large part upon the studies of Bollman, Mann,
and Magath (7, 8) and is now widely accepted. With the relation of amino acids to the formation of urea in view, amino acid formation has been measured

TABLE VI

<table>
<thead>
<tr>
<th>Oxygen 95 per cent</th>
<th>Immediate</th>
<th>After 60 minutes</th>
<th>After 120 minutes</th>
<th>After 180 minutes</th>
<th>After 240 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>43.8</td>
<td>51.9</td>
<td>58.6</td>
<td>78.2</td>
<td>96.7</td>
</tr>
<tr>
<td>Oxygen 25 per cent</td>
<td>52.9</td>
<td>61.2</td>
<td>83.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air 95 per cent</td>
<td>50.7</td>
<td>67.1</td>
<td>72.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen 95 per cent</td>
<td>47.2</td>
<td>71.8</td>
<td>67.1</td>
<td>82.3</td>
<td>86.1</td>
</tr>
</tbody>
</table>

Fig. 6. Amino acid nitrogen formed by immersed liver slices with varied oxygen supply. See Table VI.

in the present experiments under conditions that promote the formation of urea in surviving liver tissue. Amino acid nitrogen in liver slices and immersion fluid has been determined by the method of Folin (9) which compares color changes of sodium β-naphthoquinonesulfonate with those produced by glycine used as a standard. Precautions recommended by Natelson (10) have been followed. The results are recorded in Table VI and shown in Fig. 6.
In the presence of oxygen 25 or 95 per cent, with air 95 per cent and with anoxia, amino acid nitrogen has increased during 4 hours of immersion. The rate of increase has not shown a constant relation to the oxygen supply. Rapid formation of urea in the presence of 95 per cent oxygen (Table V, Fig. 5) may have diminished the quantity of amino acid that has been recoverable. It is possible that autolysis may have had some part in the production of amino acids from protein. In all instances amino acids have been available for the production of urea.

**Recapitulation and Discussion**

The tissues of the body exemplified by the liver and the kidney are not anoxic as Ehrlich (3) assumed upon the basis of experiments with alizarin blue but these experiments directed attention to the low oxygen tension of the tissues. The experiments of Campbell (4, 5) gave evidence that the partial oxygen pressure in the subcutaneous tissue and in the peritoneal cavity of rabbits, guinea pigs, rats, and cats is from 20 to 30 mm. Hg and that of carbon dioxide from 40 to 50 mm. Hg. Oxygen tension of air within alveoli of the lungs in accord with widely accepted opinion is approximately 100 mm. Hg (Table 1) and in arterial blood, 95 mm. Hg. It may be assumed that oxygen tension of the tissues cannot rise above that of the oxygen brought to them by the hemoglobin of the red corpuscles in arterial blood.

Carbon dioxide 5 per cent with partial oxygen pressure of about 35 mm. Hg, has been added to all gas mixtures because it approximates that in venous blood. Air containing about 21 per cent of oxygen has partial oxygen pressure of about 150 mm. Hg which is that at which the hemoglobin of the blood is saturated with oxygen, whatever, within physiological limits, the partial pressure of carbon dioxide (6). In normal human subjects Looney and Jellenek (11) found the oxygen content of the blood to be 91 per cent of the calculated oxygen saturation of the hemoglobin of the blood.

Water movement in liver slices exposed to anoxia under the conditions of the present experiments has been compared with that in slices gassed with air (21 per cent oxygen) or with oxygen 95 per cent, in all instances with 5 per cent carbon dioxide.

In the presence of these three gas mixtures water enters the tissue during the first 10 minutes of immersion with great rapidity. With anoxia water continues to increase and reaches a high level (Table III, Fig. 1). Slices exposed to air, on the contrary, lose during 3 hours most of the water that has entered them. With oxygen 95 per cent, which is far beyond physiological limits, loss of water is still more rapid.

Urea formation (Table IV, Fig. 2) has had a reverse relation to water content of the tissue, being greatest with 95 per cent oxygen, less with 25 per cent oxygen, and almost absent with anoxia.
Urea formed by the liver slices is presumably derived from amino acids, in part, at least, from arginine and related substances which like electrolytes are capable of maintaining osmotic pressure within liver cells (1). By oxidation urea is formed from them and being readily diffusible leaves the cells. It is noteworthy that by this means nearly all the nitrogen of substances derived from protein is eliminated by way of the urine.

With the progress of the experiments it became evident that water movement in immersed liver slices varied not only with their oxygen supply but with the stage of gastrointestinal digestion at the time when the liver tissue was obtained. In the accompanying publication (12) it is shown that liver slices take up the greatest quantity of water both with anoxia and with oxygen when they are from animals that have received no food during 20 hours. With ingestion of food, water intake by the slices has progressively diminished and has reached its lowest level 12 hours after feeding.

With these variations in view, information about the effects of oxygen supply could be obtained only when tests were made with liver slices from the same animal. The presence of 1 per cent oxygen has caused no recognizable decrease of water intake when comparison was made with anoxia. With oxygen 3.5 per cent, water content of slices has been evidently less than that with anoxia, this level of partial oxygen pressure approximating that found by Campbell in the subcutaneous tissue and peritoneal cavity of experimental animals. Oxygen 10 per cent still further decreases the water contents of liver slices. In a zone of increasing oxygen supply from 14 to 20 per cent (95 per cent of air with 5 per cent carbon dioxide) there has been little change in water content of slices. When the water content has been measured within a range of oxygen tensions beyond physiological limits it has been found to decrease continuously in the presence of oxygen 25, 50, 70, and 95 per cent.

SUMMARY AND CONCLUSIONS

Liver slices have been immersed during periods up to 4 hours at 38°C. in Krebs-Ringer solution with bicarbonate buffer and exposed to varied oxygen supply in the presence of carbon dioxide equal to that of venous blood. Water movement, urea, and amino acid formation by the liver tissue have been measured.

Water contents of surviving liver tissue diminishes with increased oxygen supply, but during life the maximum limit of oxygen is determined by that brought by the arterial blood and has an approximate partial pressure of 100 mm. Hg.

Urea formation by liver slices is increased by increased oxygen supply but does not occur with anoxia.

Osmotic pressure within liver cells is maintained in part by amino acids and related substances, and in part by electrolytes. Diminished osmotic pressure
and loss of water is explainable by oxidation of nitrogenous substances with formation of urea which leaves the cells. These changes within a limited range of variation are adjustable to functional needs.

BIBLIOGRAPHY