A PROCEDURE FOR DETERMINATION OF THE RESPIRATION OF AVASCULAR AND POORLY VASCULARIZED TISSUE MEMBRANES*

BY JOHN ESBEN KIRK, PER FROM HANSEN, POUL G. EFFERSØE, AND KURT IVERSEN

(From the Division of Gerontology, Washington University School of Medicine, St. Louis, Missouri)

(Received for publication, November 16, 1953)

In a recent publication (1) from this laboratory a procedure for determination of the respiration of tissue homogenates was reported. In the present communication the application of the technique to the determination of the respiration of tissue membranes is described.

In the homogenate method the respiration is determined by measuring the changes in the oxygen and carbon dioxide content of the homogenate. In the application of the procedure to tissue membranes the changes in the gas concentration of the medium in which the membrane is suspended are determined instead. The QO₂ and R. Q. of the tissue are calculated from the observed changes in the gas concentration of the medium, the fluid volumes present in the syringe during the experimental periods, and the dry weight of the tissue.

Procedure

Procedure B of the homogenate method as previously described (1) is used. Only those features by which the membrane technique differs from the homogenate procedure will be described.

Apparatus—Besides glass syringes of 100 and 50 ml. capacity, syringes of 30, 20, and 10 ml. volume are occasionally required. The smaller syringes are preferred for experiments in which the tissue membrane is suspended in a small volume of medium, because the finer graduation of the syringe makes possible a more exact recording of the fluid volume remaining after each sample withdrawal.

For some membranes the use of a frame is necessary to keep the tissue distended. The authors have used a ring, 25 mm. in diameter, consisting of one turn of 1 mm. platinum wire and provided with four platinum points projecting from its periphery. The ring is pliable and sterilizable in a flame. Because the ring prevents the membrane from obstructing the out-

* The investigation was supported in part by a research grant (No. PHS-891) from the National Heart Institute of the National Institutes of Health, United States Public Health Service.
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flow from the syringe, the use of saddle chips is unnecessary. With some membrane preparations, for instance cylindrical arterial segments, the use of a ring is not necessary.

Reagents—The reagents are the same as those used in the homogenate method.

Preparation of Medium and Tissue Membrane for Experiment—The buffer medium is heated to 40° and aerated with oxygen for 2 minutes as described in the homogenate method. The membrane is isolated with sterile instruments, weighed, and, if necessary, attached to the platinum ring. It is then placed in the barrel of the glass syringe. It is convenient to store the sterile wrapped syringes in the thermostat at 37° until immediately before use. Blood adhering to the surface of the membrane is removed by rinsing the membrane placed in the syringe barrel with successive 2 ml. portions of the medium. The desired amount of medium is then poured into the barrel and the plunger inserted. Any gas present is ejected, and the exposed part of the plunger and the upper part of the barrel are wrapped with sterile plastic surgical drape. The syringe is then placed in the thermostat at 37° and is rotated slowly along its axis, 15 to 30 minutes being allowed for temperature and diffusion equilibrium to become established before withdrawal of the first sample for analysis.

Analytical Technique—The analytical technique is the same as that described for the homogenate method except that, after each transfer of a sample to the Van Slyke extraction chamber, the volume of solution remaining in the syringe is recorded. In experiments in which the membrane is suspended in a small volume of medium, 0.5 to 1.0 ml. of the ferricyanide reagent and 1.5 to 1.0 ml. of the medium, respectively, may be employed for each analysis. In such cases the 2 ml. graduation mark of the extraction chamber is used for measuring out the sample of medium.

Permissible Membrane Thickness—In the application of the procedure to tissue membranes it is necessary to ascertain that the oxygen content of the medium does not fall below the value dictated by the thickness and respiratory rate of the membrane. An equation for permissible thickness of tissue slices for given values of tissue respiration and oxygen content of the medium has been established by Warburg (2).

It was found in the present study in parallel experiments on preparations of dog and human aortas (membrane thickness 1.1 to 1.7 mm., QO₂ 0.04 to 0.15) that the same respiratory rate was obtained when the tissue was suspended in samples of buffer medium containing, respectively, 1.6 to 1.9 and 0.8 to 0.9 volumes per cent of oxygen.

Time Required for Establishment of Diffusion Equilibrium—The time required for establishment of diffusion equilibrium between compounds contained in the medium and the membrane can be calculated from the diffu-
sion coefficients of the compounds for the tissue and the thickness of the membrane by the equation given by Hill (3). Determinations of diffusion coefficients for human aortic tissue at 37° according to the method of Kirk and Johnsen (4) revealed average values for oxygen, carbon dioxide, bicarbonate, and lactate of 0.00051, 0.00035, 0.00014, and 0.00011, respectively (5). According to Hill's equation, for a membrane 1 mm. thick the time required for 93 per cent equilibrium would be 4.9 minutes for oxygen, 7.2 minutes for carbon dioxide, 17.7 minutes for bicarbonate, and 22.7 minutes for lactate.

Relation between Size of Tissue Sample and Volume of Medium—Experiments were performed in which similar sized samples from the same aorta were suspended in widely varying volumes of medium. No significant difference in respiratory intensity was found with volumes of medium varying between 10 and 70 ml. per gm. of fresh arterial tissue. Since a reduction of $Q_{O_2}$ with increasing medium volume has been reported for several other tissues (6), the permissible medium volume per weight unit of tissue should be established for each type of membrane investigated.

Sensitivity of Method—With the use of large tissue samples and 9 ml. aliquots of medium for gasometric analysis the procedure permits measurement of $Q_{O_2}$ rates as low as 0.01.

Error Introduced by Gradual Release of Blood from Membrane to Medium—A gradual release of blood from the tissue to the medium during an experiment by increasing the oxygen content of the medium will result in the registration of a too low oxygen consumption by the membrane. It was found in experiments on rat diaphragms that the release of blood, even from carefully rinsed diaphragms, was sufficient to introduce an appreciable error. For this reason the method cannot be recommended for use with highly vascularized membranes.

Comparison of Results Obtained with Present Procedure and with Warburg Technique—In parallel experiments on the oxygen consumption by the mucous membrane of the rabbit urinary bladder a satisfactory agreement was found between values obtained with the present method and the Warburg technique. Thus the determination of the respiration by the syringe procedure gave a $Q_{O_2}$ value of 0.51 whereas the mean $Q_{O_2}$ of measurements with the Warburg method was 0.48.

**EXPERIMENTAL**

The protocol for an experiment illustrating the method for calculation of the tissue respiration is given in Table I. In recording the fluid volume present in syringe during each experimental period a correction is made for the volume of the tissue sample, the dry weight of which is subtracted from the observed fluid volume.
In a control experiment performed with the buffer medium alone over a 3 hour period no change in the oxygen or carbon dioxide concentrations of the medium was found.

**TABLE I**

*Protocol and Calculation of Tissue Respiration*

The experiment was conducted on 615 mg. (dry weight) of thoracic aorta 2 days after the death of a 67 year-old human male subject. The medium employed was Krebs' phosphate buffer with 0.2 per cent glucose. Sterility, as determined by bacterial culture, was maintained throughout the experiment. Sample of medium used for analysis = 9.0 ml.; \( a = 0.5; S = 10.0 \) ml.; temperature = 24°.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>( p_1 )</th>
<th>( p_2 )</th>
<th>( p_3 )</th>
<th>( p_3 - p_2 )</th>
<th>( CO_2 )</th>
<th>( CO_2 - CO_1 )</th>
<th>( O_2 )</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>442.7</td>
<td>429.2</td>
<td>147.4</td>
<td>13.5</td>
<td>0.113</td>
<td>281.8</td>
<td>1.899</td>
</tr>
<tr>
<td>106</td>
<td>437.7</td>
<td>397.2</td>
<td>146.0</td>
<td>40.5</td>
<td>0.340</td>
<td>251.2</td>
<td>1.693</td>
</tr>
<tr>
<td>186</td>
<td>432.6</td>
<td>365.8</td>
<td>147.4</td>
<td>66.8</td>
<td>0.561</td>
<td>218.4</td>
<td>1.472</td>
</tr>
</tbody>
</table>

Calculation of respiration in first period, time = 106 minutes. Volume of medium in syringe, 58.5 - 0.6 = 57.9 ml. Decrease in oxygen concentration of medium, 1.899 - 1.693 = 0.206 volume per cent. Oxygen consumed, 0.206 \( \times \) 0.579 = 0.119 ml. \( Q_{O_2} = (119 \times 60)/(615 \times 106) = 0.11 \). Increase in carbon dioxide concentration of medium, 0.340 - 0.113 = 0.227 volume per cent. R. Q. = (0.227/0.206) = 1.10.

Calculation of respiration in second period, time = 80 minutes. Volume of medium in syringe, 35.0 - 0.6 = 34.4 ml. Decrease in oxygen concentration of medium, 1.693 - 1.472 = 0.221 volume per cent. Oxygen consumed, 0.221 \( \times \) 0.344 = 0.076 ml. \( Q_{O_2} = (76 \times 60)/(615 \times 80) = 0.09 \). Increase in carbon dioxide concentration of medium, 0.561 - 0.340 = 0.221 volume per cent. R. Q. = (0.221/0.221) = 1.00.

**DISCUSSION**

The present procedure possesses the advantage of a much higher sensitivity than the Warburg method and is therefore particularly suitable for investigations on membranes having a low respiratory rate. The method may also be applied to avascular organs like the animal lens. The further advantages of the syringe method have been discussed previously (1).

For the study of rapidly respiring membranes the syringe procedure has the disadvantage that prolonged experiments cannot be undertaken without reaeration of the medium, because the tissue respiration quickly reduces the oxygen concentration of the medium below the level required for supply of the central sections of the membrane with oxygen. The fact that most rapidly respiring membranes are highly vascularized precludes in itself the use of the present method for such membranes.

**SUMMARY**

The procedure of Kirk and Hansen for measurement of the respiration of tissue homogenates has been applied to the determination of the respiration of avascular and poorly vascularized tissue membranes.
The method is particularly suitable for investigations on slowly respiring membranes.

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