AN APPARATUS FOR THE STUDY OF RESPIRATORY QUOTIENT AND BASAL METABOLISM OF MICE

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During the course of some investigations in this laboratory, it became necessary to ascertain the respiratory quotient and basal metabolism of mice. Therefore, a study was made of an apparatus and procedure which could be readily applied to a large number of animals, and would yield precise results.

Apparatus for determining respiratory quotients fall into two classes, the open-circuit type and the closed-circuit type. In the former, a stream of dry air freed from carbon dioxide is passed through a chamber containing the animal. The water vapor and carbon dioxide in the outgoing air are absorbed and weighed. At the same time, the loss in weight of the animal and the weight of its container are noted. The amount of oxygen used is then determined indirectly as the difference between the combined weights of water and carbon dioxide given off and the loss in the weight of the animal. Considerable precaution must be taken in weighing the absorption vessels and animal chamber. Haldane was among the first to apply this procedure to the study of respiratory exchange of small animals, so that the method is commonly known by his name. This apparatus was also used by Pembry in studying the rate of respiration of mice at various temperatures, and by Moog for determining the respiratory quotient of guinea pigs. It has been somewhat modified and greatly complicated by Murschauser, who removed the carbon dioxide by means of an elaborate system of absorption bottles containing barium hydroxide solution, and determined the oxygen consumed by analysis of samples of the expired air. More recently, Aszodi has used a simple apparatus of this type for the study of induced hibernation in white mice. Several experiments in which the oxygen consumed was ascertained directly as well as indirectly showed that the values obtained by the two methods agreed to within 2

1 Haldane, J. S., J. Physiol., 1891, 13, 419.
4 Murschauser, H., Biochem. Z., 1912, 42, 262.
5 Aszodi, Z., Biochem. Z., 1921, 113, 70.
per cent. Aszodi's apparatus has been applied to the metabolism of white rats by Goto, Asada, and Händel and Tadenuma. A very simple continuous-flow apparatus has been described by Artundo for use with rats and rabbits in which the respiratory exchange is determined by analysis of samples of the expired air by means of a Haldane gas analysis apparatus.

In the closed-circuit type of apparatus, the respired air is passed through an absorbent to remove the carbon dioxide and then returned to the respiration chamber to be used over again. Oxygen is supplied as needed to replace that taken up by the animal. Thus, the oxygen consumed is measured directly by the quantity required to maintain constant pressure in the system. The amount of carbon dioxide given off is obtained by weighing the absorption vessels or by titration. An apparatus of this type was first applied to the study of small animals by Regnault and Reiset. Zuntz has described an exceedingly complicated apparatus operating on this principle which was later modified by Zuntz and Oppenheimer. Elsas studied the respiratory exchange of frogs, using an apparatus in which air was circulated by a motor-driven pump, and the carbon dioxide absorbed in barium hydroxide solution was determined by titration. The oxygen was measured as introduced from a gasometer and checked by analysis of samples of the respired air. The experimental period extended over 20 to 24 hours. He considered the average error to be about 2 per cent. The same apparatus was used by Lesser for the determination of the respiratory quotients of mice. He reported that the error in the quotients obtained did not exceed 0.02. Hildebrandt used a similar apparatus for the study of the respiration of white rats. A model described by Benedict for use with rabbits and guinea pigs includes special provision for recording movement of the animal and for introducing food. It is designed for use over a period of 24 hours or longer. A very elaborate apparatus for experiments lasting a week or more has been constructed by Kolls and Lovenhart. Rubner has described a comparatively simple device for use with animals weighing from 20 to 60 grams. Air is circulated by means of a rotary

7 Asada, K., Biochem. Z., 1923, 143, 387.
12 Zuntz, N., and Oppenheimer, C., Biochem. Z., 1908, 14, 361.
15 Hildebrandt, F., Arch. exp. Path. u. Pharm., 1922, 92, 68.
pump. Oxygen is supplied by electrolysis, the volume added being determined by measuring the volume of hydrogen evolved at the cathode. The duration of an experiment is about 24 hours. Other apparatus for use with small animals have been described by Foster and Sundstroem,\textsuperscript{19} Wesson,\textsuperscript{20} and Schoeller, Gehrke, and Michael.\textsuperscript{21} Fridericia\textsuperscript{22} has described a closed-circuit apparatus in which the oxygen consumed can be determined by two methods, thus obtaining a double check on this value. Cori and Cori\textsuperscript{23} have used the apparatus of Fridericia in a study of the metabolism of rats. Agreement between the values of direct and indirect oxygen determinations averaged 1.4 per cent. They consider the value for the respiratory quotient to be accurate to ± .008.

Although many of the apparatus cited above are applicable to the study of the metabolism of mice, none are particularly adapted to rapid routine manipulation. The difficulties encountered in keeping a large circulatory system perfectly air tight have been emphasized by many workers. Any procedure involving methods of exact gas analysis requires particular care and is very time consuming, as is true of methods involving accurate weighing of gas absorption vessels. An attempt to develop a procedure which will avoid these disadvantages, and at the same time provide a method for the measurement of the respiratory quotient with sufficient accuracy, has resulted in the apparatus and technique described in this paper.

The apparatus (Fig. 1) consists of a closed glass chamber (A) of about 500 cc. capacity, in which a wire cage (B) is suspended. The carbon dioxide given off by the animal is absorbed by an N/20 solution of barium hydroxide. This solution is introduced into the chamber from burette (C) at the beginning of the experiment. At the conclusion of the experiment, the excess barium hydroxide is titrated with an N/50 solution of oxalic acid contained in burette (D). An air-propelled fan and glass paddle mounted on the same shaft circulate the air and thoroughly stir the liquid. The shaft of the stirrer enters the chamber through a mercury seal (E). The chamber is ventilated before and after the experiment by aspirating a current of air through tubes (F) and (G). Variations in the pressure during the run are

\textsuperscript{22} Fridericia, L. S., \textit{Biochem. Z.}, 1913, \textit{54}, 92.
recorded on the manometer (H). When the chamber is closed, the animal consumes oxygen and the pressure in the chamber is reduced. Compensation is made by running in an equivalent volume of barium hydroxide solution so that the amount of oxygen consumed is measured by the volume of barium hydroxide solution required to maintain atmospheric pressure within the chamber. The volume of carbon dioxide given off during the same period is calculated from the titration. The respiratory quotient is then obtained by dividing this volume of carbon dioxide by the volume of oxygen consumed. Proper functioning of the apparatus can be checked at any time by running a blank experiment in which a measured amount of pure, dry carbon dioxide is passed into the chamber. Capillary stop-cock (I) is provided for this purpose. The brass plate which carries the cage, together with all inlets and outlets to the chamber, is fixed rigidly to an iron stand which also supports the burettes and reservoirs. The removable wire cage (Fig. 2) is provided with a sliding floor for the introduction of the animal. The glass chamber rests on a brass plate which is clamped to the fixed plate by thumb screws. The fixed plate carries a rubber gasket which insures an air-tight seal. The chamber and reservoir (J) are immersed in a water thermostat. The purpose of this reservoir is to eliminate temperature changes in the barium hydroxide solution when it is introduced into the chamber. Absolute quiescence of the animal throughout the experiment is assured by an intraperitoneal injection of "luminal sodium."

Experimental Procedure

The animal is injected intraperitoneally with 0.10 cc. of a 2 per cent solution of "luminal sodium," and immediately placed in the wire cage which is then suspended under the fixed plate. Since, during the experiment, the air of the chamber is in contact with a dilute aqueous solution, provision must be made for saturating it with water vapor during the preliminary period. Otherwise the vapor pressure, which would develop as soon as the chamber should be closed, would introduce error into the oxygen determination. To this end, 5 cc. of water are placed in the chamber together with a few drops of phenolphthalein indicator necessary for the subsequent titration. The chamber is then clamped firmly into place. The fan is started, and the suction adjusted so as to provide a current of air at the rate of about 1 liter per minute. For the reasons just mentioned, the air used for ventilation must not only be at the same temperature as the thermostat but must also be saturated with water vapor. The incoming air, therefore, circulates...
through a copper coil and humidifying device immersed in the bath. The temperature and humidity of the incoming air may easily be checked by closing the chamber and observing any changes in the manometer. About 30 minutes are required to reach thermal equilibrium. However, the actual run is not started until one hour has elapsed from the time of the injection, so that the animal will be thoroughly under the influence of the drug. With the current of air still passing through the chamber, about 30 cc. of the barium hydroxide solution are introduced, stop-cock (G) is turned to divert the suction to the open air, and stop-cock (F) is closed, thus sealing the chamber. The time of closure is recorded and the barium hydroxide burette is read. As the oxygen is consumed by the animal, the pressure in the chamber decreases, as indicated by the manometer. Barium hydroxide solution is run in as required to restore atmospheric pressure. At the end of about 30 minutes, the time is again recorded, stop-cock (F) is opened, suction is restored, and the excess barium hydroxide titrated rapidly with oxalic acid. The volume of oxygen consumed is given directly by the volume of barium hydroxide solution introduced during the run. From the titration data, the temperature of the bath, and the barometric pressure, the volume of carbon dioxide given off by the animal is computed. Data and calculations from a typical experiment follow.

<table>
<thead>
<tr>
<th>Weight of animal</th>
<th>23.3 gm.</th>
<th>Normality of Ba(OH)$_2$</th>
<th>0.0491</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature of bath</td>
<td>28°C.</td>
<td>Normality of H$_2$C$_2$O$_4$</td>
<td>0.0204</td>
</tr>
<tr>
<td>Barometric pressure</td>
<td>760 mm.</td>
<td>Duration of experiment</td>
<td>28 minutes</td>
</tr>
</tbody>
</table>

- Ba(OH)$_2$ introduced at start: 30.00 cc.
- Ba(OH)$_2$ added to compensate for oxygen consumed: 21.55 cc.
- Total Ba(OH)$_2$ added: 51.55 cc.
- Volume of H$_2$C$_2$O$_4$ equivalent to 51.55 cc. of Ba(OH)$_2$: 123.72 cc.
- H$_2$C$_2$O$_4$ required to titrate excess Ba(OH)$_2$: 59.35 cc.
- H$_2$C$_2$O$_4$ equivalent to CO$_2$ absorbed: 64.37 cc.

Volume of CO$_2$ absorbed at standard conditions as calculated from above: 14.64 cc.
Volume of CO$_2$ absorbed at 760 mm. and 28°C: 16.14 cc.
Volume of O$_2$ consumed at 760 mm. and 28°C (vol. Ba(OH)$_2$ added): 21.55 cc.
Respiratory quotient = $\frac{16.14}{21.55} = 0.749$

**Standardisation of the Apparatus**

The accuracy of the apparatus is dependent upon the extent to which the carbon dioxide absorbed represents the carbon dioxide given off by the animal during the time of the run. The degree to which this condition is fulfilled may be tested by introducing a known
volume of pure carbon dioxide under circumstances which simulate as nearly as possible the conditions of the experiment.

TABLE I

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Rate of introduction of CO₂ (cc. per minute)</th>
<th>Volume of CO₂ Introduced</th>
<th>Volume of CO₂ Titrated</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.47</td>
<td>15.00</td>
<td>14.91</td>
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<tr>
<td>2</td>
<td>0.48</td>
<td>15.00</td>
<td>15.06</td>
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<td>3</td>
<td>0.51</td>
<td>15.30</td>
<td>15.24</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
<td>20.00</td>
<td>20.20</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>20.00</td>
<td>20.20</td>
</tr>
<tr>
<td>6</td>
<td>1.1</td>
<td>16.20</td>
<td>16.70</td>
</tr>
<tr>
<td>7</td>
<td>1.8</td>
<td>21.60</td>
<td>21.50</td>
</tr>
<tr>
<td>8</td>
<td>1.9</td>
<td>20.00</td>
<td>20.20</td>
</tr>
<tr>
<td>9</td>
<td>3.2</td>
<td>20.00</td>
<td>19.25</td>
</tr>
</tbody>
</table>

TABLE II

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Animal</th>
<th>Weight (grams)</th>
<th>Duration of run (minutes)</th>
<th>O₂ consumed (cc)</th>
<th>CO₂ evolved (cc)</th>
<th>R.Q. (CO₂/O₂)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>19.30</td>
<td>37</td>
<td>20.00</td>
<td>14.10</td>
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<tr>
<td>2</td>
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<td>20.00</td>
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<tr>
<td>3</td>
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<td>20.20</td>
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<tr>
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<td>20.20</td>
<td>14.27</td>
<td>0.706</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
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<td>20.30</td>
<td>14.10</td>
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<td>20.60</td>
<td>14.32</td>
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</tr>
<tr>
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<td>4</td>
<td>26.40</td>
<td>31</td>
<td>20.05</td>
<td>14.50</td>
<td>0.725</td>
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<tr>
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<td>20.00</td>
<td>14.17</td>
<td>0.708</td>
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<tr>
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<td>20.50</td>
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<td>14.04</td>
<td>0.695</td>
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<tr>
<td>11</td>
<td>6</td>
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<td>36</td>
<td>20.05</td>
<td>14.62</td>
<td>0.729</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>22.00</td>
<td>35</td>
<td>20.00</td>
<td>14.62</td>
<td>0.731</td>
</tr>
</tbody>
</table>

Average ......................................................... 0.710

The apparatus is arranged in the thermostat and a stream of carbon dioxide from a gas burette is passed into the chamber through the capillary stock-cock (I). The rate of introduction of the gas is adjusted to duplicate as nearly as possible
the rate at which carbon dioxide is normally expired by a mouse, as determined in previous experiments. In order to be certain that variations in the rate of production of carbon dioxide, which might be expected in normal animals, were without appreciable effect upon the validity of the data, carbon dioxide was introduced at rates varying from 0.5 to 3 cc. per minute. The stream of carbon dioxide is allowed to pass into the chamber during a preliminary period of about 20 minutes. 50 cc. of barium hydroxide solution are run into the chamber, this quantity being approximately the total amount used in a determination upon an animal. The chamber is then closed. The stream of carbon dioxide is continued until about 15 to 20 cc. have been introduced. Ventilation is reestablished and the excess barium hydroxide titrated. A comparison of the carbon dioxide absorbed with the carbon dioxide introduced, as read on the gas burette, serves as an indication of the accuracy of the apparatus. Data obtained in this manner are given in Table I.

The operation of the apparatus was further checked by determining the respiratory quotients of mice which had been fasting for 36 hours. It has been shown that fasted rats give a respiratory quotient of 0.71 to 0.72, and Benedict has recommended their use for the standardization of apparatus employed in the study of metabolism of small animals. Duplicate runs were made in each case. Data obtained are given in Table II.

DISCUSSION

The apparatus and procedure here outlined are, in some respects, distinct departures from the methods heretofore employed. In the first place, the animal is confined in a chamber where the atmosphere is saturated with water vapor. Although it has been shown that above 25°C. the effect of increased humidity is to raise the metabolic rate, there is little reason for believing that it would have any pronounced effect on the respiratory quotient. Values for the respiratory quotient of normal animals determined in this apparatus appear to confirm this inference.

In other apparatus of this type, the composition of the air is kept practically constant throughout the experiment by introducing pure oxygen to replace that consumed. This necessarily involves complications in the apparatus which can be avoided if the oxygen content be allowed to decrease within certain limits. It has already been

shown\textsuperscript{28, 27, 28, 29} that a decrease in the oxygen content of respired air is without noticeable effect upon respiration as long as it does not fall below 10 to 12 per cent. In the present procedure, the concentration of oxygen in the chamber is never less than 15 per cent, so that the metabolism of the animal should at no time be affected by oxygen want.

The success of the carbon dioxide determination depends upon the extent to which the amount of carbon dioxide, computed from the titration, represents the amount of carbon dioxide given off by the animal during the time of the experiment. If air is passed through the chamber at the rate of one liter per minute, the concentration of carbon dioxide, initially present, will be from 0.5 to 1 cc. per liter. Experiments in which pure carbon dioxide was introduced into the chamber indicated that with this initial concentration the carbon dioxide was taken up by the barium hydroxide as fast as it was introduced, so that, at the close of the run, the concentration of carbon dioxide was practically unchanged. Therefore, the carbon dioxide present at the beginning is compensated for by the same amount of carbon dioxide left unabsorbed at the end of the run. This being the case, the carbon dioxide absorbed will be an accurate measure of the carbon dioxide given off by the animal during the time of an experiment.

The volume of the chamber necessarily imposes certain limits upon the total amount of fluid introduced. Although the accuracy of a titration is favored by the use of dilute solutions, it is essential that the barium hydroxide solution be of a sufficient concentration to produce rapid absorption of carbon dioxide. It was found that an N/20 solution best fulfilled these requirements. The limited space available for titration did not make it feasible to use an acid solution of a greater dilution than N/50.

The accuracy of the carbon dioxide determination will be affected by the speed at which the barium hydroxide solution is introduced at the beginning of the run, and the time required to titrate the excess

\textsuperscript{28} Friedländer, C., and Herter, E., \textit{Z. A. physiol. Chem.}, 1879, 3, 19.
\textsuperscript{28} Terray, P., \textit{Pflüger's Arch.}, 1896, 65, 393.
\textsuperscript{29} Durig, A., \textit{Arch. f. Physiol., Suppl.}, 1903, p. 209.
barium hydroxide at the end of the run. However, by the exercise of reasonable precaution, error from this source should be negligible.

From a study of the data in Table II, it appears that the probable error of a single observation is 0.015, and the probable error of the arithmetical mean is 0.004. These data, together with the results obtained in the standardization of this apparatus with pure carbon dioxide, indicate that the values for the respiratory quotients obtained are accurate within 0.02. The mean value obtained from a series of such observations should be readily brought to an accuracy of 0.01.

CONCLUSIONS

A simple apparatus has been developed for the study of the respiratory quotient and basal metabolism of mice. Data are given which indicate that the values for the respiratory quotient obtained are accurate to within 0.02. The apparatus is especially designed for rapid, routine manipulation.