SOME THERMOANALYTIC STUDIES OF ORGAN AND WHOLE ANIMAL RESPIRATION

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INTRODUCTION

These studies are an attempt to obtain comparative information about the thermal behavior of the different organs in an animal body. From these data, and from similar data about the thermal behavior of the whole animal, it is possible to draw certain inferences regarding the conditions which govern the oxygen consumption of tissues. Such inferences are the chief interest of the present paper. More extensive investigations of this sort, however, might also greatly clarify problems of the individual metabolism in tissues. This last question we shall touch upon briefly.

Technical Details

All the present experiments were carried out on tissues of the winter variety of Rana pipiens. The organs used were liver, kidney, and external and internal oblique muscles. Tissue slices were made free-hand with an ordinary razor blade (1); after some practice these were consistently turned out with a thickness of less than 0.2 mm. These slices were suspended in unbuffered Locke’s solution.* It was established by preliminary experiments (see below) that with the solution and amounts of tissue used (< 50 mg.), the increasing acidity of the system was a negligible factor for a period of at least 2 hours. (Readings were subsequently taken for a period of 1 hour only.) The respirometers used were simple manometric systems (American Instrument Co.). Temperature control good to 0.1°C. was obtained. “Dry weight” was determined as the constant weight attained after drying in an oven at 86°C.

The Results of Preliminary Experiments.—It is, of course, an essential assumption in the measurements of tissue respiratory rates that the determined rate be independent of the duration of the run or of the excision time. This is not the case if the tissue has a high cytolytic rate (rapid negative acceleration of the oxygen consumption). In order to investigate the cytolytic behavior of these tissues, the curve of $Q_{O_2}$ vs. time was obtained in a large number of experiments lasting an average of 8 hours. In each case it was found that the tissues exhibited a constant $Q_{O_2}$, i.e. $dQ/dt = 0$, for at least 2 hours after the

\[
\begin{align*}
\text{NaCl} & : 7.0 \text{ gm. li}^{-1} \\
\text{CaCl}_2 & : 0.316 \\
\text{KCl} & : 0.189 \\
\text{Glucose} & : 1.000
\end{align*}
\]
beginning of the experiment or 2 hours and 25 minutes after excision. On the basis of this fact it was decided to take total oxygen consumption during the 1st hour as the value of the $Q_{O_2}$. At all temperatures the second differences of the pressures ($\Delta^2 p$) were always checked to establish the absence of a negative acceleration. In no case was this detected during the time of the run. This is, of course, in striking contrast to the behavior of mammalian tissues.

### TABLE I

**$Q_{O_2}$ Values for Various Organs at Different Temperatures**

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>25.6°</strong></td>
<td>1.86</td>
<td>1.93</td>
<td>0.324</td>
</tr>
<tr>
<td>(10)*</td>
<td></td>
<td>(5)</td>
<td>(9)</td>
</tr>
<tr>
<td><strong>30.0°</strong></td>
<td>0.782</td>
<td>2.72</td>
<td>0.282</td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td><strong>20.0°</strong></td>
<td>1.18</td>
<td>1.58</td>
<td>0.46</td>
</tr>
<tr>
<td>(7)</td>
<td></td>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td><strong>15.0°</strong></td>
<td>0.606</td>
<td>1.385</td>
<td>0.389</td>
</tr>
<tr>
<td>(5)</td>
<td></td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td><strong>10.0°</strong></td>
<td>0.467</td>
<td>1.01</td>
<td>0.279</td>
</tr>
<tr>
<td>(5)</td>
<td></td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td><strong>5.0°</strong></td>
<td>0.310</td>
<td>0.816</td>
<td>0.322</td>
</tr>
<tr>
<td>(5)</td>
<td></td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td><strong>0.0°</strong></td>
<td>0.29</td>
<td>0.58</td>
<td>0.27</td>
</tr>
<tr>
<td>(5)</td>
<td></td>
<td>(5)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses signify No. of determinations used in calculating the mean given.
FIG. 1. Oxygen consumption in mm.³ hr.⁻¹ mg.⁻¹, for liver slices (*Rana pipiens*) between 0 and 30°C. The measures were made at approximately 5° intervals.

The initial constancy of the QO₂ has the corollary that the acidity of the tissue did not undergo a non-physiological displacement, for such a displacement would necessarily bend the time curve away from linearity. Thus the absence of a buffer was justified.

It might be remarked in passing that much of interest could probably be
FIG. 3. Oxygen consumption in mm.² hr.⁻¹ mg.⁻¹, for slices of the oblique abdominal muscles (Rana pipiens) between 0 and 30°C. The measures were made at approximately 5° intervals.

FIG. 4. Oxygen consumption of organs and animal referred to oxygen consumption for the same organ or animal at 0°C. (Rana pipiens). The ratios are given at approximately 5° intervals.
gained by performing a rational analysis of the time curves. The constants of such curves would serve as comparative indices for the tissues.

**FIG. 5.** Arrhenius plots of log (oxygen consumption) vs. the reciprocal of the absolute temperature. Straight lines have been oriented through the points in order to secure approximate values of \( \mu \).

**RESULTS**

The results of all experiments are summarized in Table I, in which entries are made so that for a given tissue (at a given temperature) there are at least five determinations on different animals. The means of readings are then used variously in a series of graphs: (1) \( Q_0 \) vs. Centigrade temperature. (2) A comparison of the various tissues and of the whole animal, using the dependent variable, \( Q/Q^o \), where \( Q^o \) is the oxygen consumption at 0°C. (3) Plots of log \( Q_0 \) vs. \( 1/T \) for the various tissues and for the whole animal. (4) \( Q_0 \) vs. time
after excision at 298°K, for liver, muscle, and kidney—a representative graph of preliminary experiments. Figs. 1, 2, and 3 contain graph (1) for liver, kidney, and muscle respectively. Figs. 4, 5, and 6 contain graphs (2), (3), and (4) respectively.

Fig. 6. Total oxygen mg.−1 consumed up to time t vs. t. 298°K. The slope of this integral curve gives the instantaneous rate of oxygen consumption.

DISCUSSION

The following facts expressed in the results would seem to merit attention:

1. Nature of the Curve: $Q_{O_2}$ vs. Centigrade Temperature.—Throughout the temperature range to which the animal might be exposed in nature, $Q_{O_2}$ is an ascending function, although for striated muscle this ascent is very gentle. With regard to this particular case, however, it must be remembered that in the animal the muscle is always in a state of tonus, and that the steady state chemical system of such contractions will generally have a different temperature coefficient from that of absolutely resting muscle, since the two systems differ qualitatively. The $Q_{O_2}$ of liver passes through a definite maximum near 25°C. The drop subsequent to 25° is rather rapid, and this fact suggests that it is probably due to deteriorative reactions.\footnote{Through the kindness of Mr. F. Kreutzer, a histological study of all these tissues exposed to temperatures of 10° and 30° for the length of time of a run has been carried out. Unmistakable degenerative changes in both nuclei and cytoplasm were evident at 30° and absent at 10°.}

The intact animal itself
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does not stand such temperatures very well. Muscle $Q_{O_2}$, like that of liver, appears to pass through a maximum, in the neighborhood of 20°C. It is, of course, tempting to correlate the presence of maxima in this range with the common feature in the metabolism of the two cases, namely, the reactions of glycogen. Kidney $Q_{O_2}$, on the other hand, rises continuously in the temperature range studied, and, moreover, in a manner (as will be shown later) characteristic of the velocity variation of a single reaction system. The natural inference is that oxygen is consumed in the undisturbed portion of the reaction system, while the heat deterioration affects a separate portion. The die-away (deterioration) of cells is known to be more rapid at the higher temperatures; consequently it must be that the kidney possesses thermostable energy stores

**TABLE II**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Whole animal (Krogh)</th>
<th>Respiration through skin (Pitter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>1.08</td>
<td>1.41</td>
<td>1.19</td>
<td>1.5</td>
<td>4.3</td>
</tr>
<tr>
<td>10</td>
<td>1.61</td>
<td>1.74</td>
<td>1.03</td>
<td>3.12</td>
<td>10.5</td>
</tr>
<tr>
<td>15</td>
<td>2.09</td>
<td>2.39</td>
<td>1.44</td>
<td>5.5</td>
<td>15.8</td>
</tr>
<tr>
<td>20</td>
<td>4.07</td>
<td>2.72</td>
<td>1.70</td>
<td>9.6</td>
<td>21.0</td>
</tr>
<tr>
<td>25.6</td>
<td>6.4</td>
<td>3.34</td>
<td>1.20</td>
<td>19.2</td>
<td>24.3</td>
</tr>
<tr>
<td>30</td>
<td>2.70</td>
<td>4.70</td>
<td>1.04</td>
<td>44.0</td>
<td>29.0</td>
</tr>
</tbody>
</table>

in sufficient amount to maintain the thermostable portion of the reacting system for a period of at least an hour and a half.

**II. Comparisons of the Tissues and Animal (See Table II).**—The striking comparative fact regarding these experiments is that the organs differ radically in their thermal behavior, both among themselves and with respect to the whole animal. This is well illustrated in the second group of plots wherein the oxygen consumptions are expressed as per cents of standard (0°C.) values. At any one temperature, $Q$, $Q/Q^0$, and $dQ/dT$ will generally be different, and the maxima will fall at different values of $T$.

These observations indicate the fallacy of using data on the thermal behavior of the whole animal too enthusiastically. Even when nervous effects are disregarded the total oxygen consumption is incomplete metabolic information. A point of more fundamental importance is also suggested by these inter-organ differences. With some reservation, the amount of oxygen consumed by a cell of these three types can be taken as a measure of the energy input of the cell. It would now be exceedingly interesting to obtain a similar measure of the energy output (e.g. in the kidney, according to the treatment of Borsook (2)), so that efficiency as a function of temperature could be ascer-
tained. It is conceivable that the efficiencies for various organs might have optima at approximately equal temperatures. The coincidence or non-coincidence of the efficiencies is of course of more significance than the coincidence or non-coincidence of respiratory maxima.

III. Arrhenius Plots.—The final consideration which we wish to undertake here is the possibility of applying Crozier’s thermal analysis to the problem: is the oxygen consumption of a given tissue limited by the availability of the gas or by the metabolic machinery of the tissue which uses up the gas? A theoretical answer to this question can be given if it is assumed that the uptake of oxygen occurs according to the scheme:

\[
\begin{align*}
\text{O}_2 \text{ in air} & \rightarrow \text{O}_2 \text{ in blood} & \rightarrow \{ \text{O}_2 \text{ metabolizing in the tissue} \}
\end{align*}
\]

Let us suppose that we know the \( \mu \) value of the over-all process (whole animal respiration) and also that of the second step (tissue respiration). If these values differ by the requisite Burton amount (3), then it follows that the \( \mu \) value of the over-all process was actually the \( \mu \) value of the first step, and therefore that the first step (availability) is the limiting process or “master reaction.” If the difference in \( \mu \) values is insufficiently large, then, according to Burton, nothing can be said regarding mastery. This view is not shared by the author,\(^3\) and here we should like to retain tentatively the original condition that if \( \mu_i > \mu_j \), then \( i \) is the master reaction of the process.

To investigate the question we must have the \( \mu \) values for the whole animal (over-all process) and for the chief respiring tissues of the animal. For the whole animal the \( \mu \) values are, according to Crozier’s (4, 5) calculations, 24,000 cals., 21,000 cals., and 11,000 cals., respectively, for Vernon’s (6), Krogh’s (7, 8), and Pütter’s (9) (respiration through the skin) experiments. With respect to the tissues it will be seen that the plots are of various types—a matter which we shall comment on below. Kidney gives a \( \mu \) value of 8,400 cals. Liver does not give a good rectilinear plot (in fact, the points fall on a rather regular curve), but a rough estimate of the characteristic is 12,000 cals. The muscle plot is highly irregular, but it is safe to say that the greatest possible value (computed from the maximum slope) is less than 5,000 cals.

Using these data in the theory, we are led to the deduction that both for kidney and for muscle the site for the master reaction in their metabolism is between the organ and the external air. In the frog the site would be at the lungs and possibly on the skin, and the limiting process would be the diffusion of oxygen through these membranes.

The case of liver is not so clear cut. Its \( \mu \) value is considerably below those

\(^3\) This matter is considered in a paper now in preparation.
for the whole animal (a difference of about 8,000 cals.), but it is practically equal to that for whole animal respiration through the skin. From this we can very tentatively conclude that whenever lung respiration predominates, the oxygen consumption of the liver is limited by the availability of gas, but that whenever skin respiration predominates no master reaction can be said to exist. It is curious to note in this connection that the $Q/Q_0$ curves for liver and for respiration through the skin are strikingly similar.

Finally, it might be remarked in passing that the form of the curve in the Arrhenius plot may turn out to be of great significance even when curvilinear (as is the case for liver in these experiments). If we accept the statistical form of the velocity constant (see, for instance, 10), it follows that by the operation of taking logarithms this function will always be split up into two terms, one of which will contain the energy of activation and $1/T$. The temperature dependence of the other term is what may decide departures of the plot from linearity.

The counsel of Professor S. F. Cook and of Mr. F. Kreutzer in connection with this work is most gratefully acknowledged.

SUMMARY

Data on the respiratory rates of frog liver, kidney, and striated muscle were obtained at various temperatures by the Warburg method. Fundamental differences in the curves of $Q_0$ vs. $T$ exist among the tissues and between the tissues and the whole animal. The Arrhenius plots of these curves show that at least for some tissues the availability of oxygen at the tissue, as limited by the diffusion of gas through the skin and lungs, governs the $Q_0$ of the tissue. Inferences are drawn regarding the comparative metabolism of the tissues and the fallacy of using whole animal $Q_0$ alone as a metabolic index.

BIBLIOGRAPHY