88. Effect of Carbon Dioxide on Conservation of Physiological Activities of Animal Tissues. I

Hyperthermic Potassium Contracture of Rat Skeletal Muscle (Musculus soleus)

By Hisateru Mitsuda, M. J. A., Yasuyoshi Azuma, and Saburo Ueno
The Foundation of Interdisciplinary Research Institute of Environmental Sciences*

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Introduction. Following Smith's description about Hickman's study on the anesthetic effects of carbon dioxide in dogs,1) many studies were made on the physiological and anesthetic effects of carbon dioxide in animals.2)-7) We investigated the differences in liver catalase activities among hibernating and non-hibernating animals, and found a significantly lower optimum temperature for catalase in the hibernating animals.8)13) It was also suggested that the lower metabolic rate of the hibernating animals was caused by a higher gas tension of carbon dioxide in the environment and the lower body temperature, so it is expected that the physiological activities of animal tissue can be preserved for a long time under higher gas tension of carbon dioxide at a lower temperature.

The present study investigated the effect of carbon dioxide and oxygen on preservation of physiological activities of isolated rat skeletal muscle, Musculus soleus (M. soleus), during potassium-induced contracture, as a model of short term preservation of isolated animal tissue that is kept at a higher temperature (42°C) corresponding to the hyperthermic temperature in animals. The temperature-time dependence of the potassium contracture could be extrapolated to those of the lower temperature according to the Q_{10} law, which has been generally accepted in the temperature-activity dependence in the living tissue.

Materials and Methods. Intact M. soleus (about 20 mg in weight) obtained from male Sprague-Dawley rats weighing 68–72 g were used. The muscle preparation was placed in a Magnus tube filled with normal Krebs-Ringer solution (pH 7.4–7.5, Table I) kept at 42°C and continuously bubbled with carbogen (CO₂ 5% + O₂ 95%). The lower end of the muscle was fixed and the upper end was attached to an isometric force transducer (Nihon Kohden TB-651T) connected to an isometric amplifier (Nihon Kohden EF-601G) and a pen recorder (YEW Type 3056) as illustrated in Fig. 1.

Resting tension was adjusted to 1.0 g and the muscle preparation was allowed to equilibrate under environmental conditions for 45 min, then a preliminary potassium contracture was induced by exchanging the solution with fresh Krebs-Ringer solution which contains 100 mM KCl, and after 15 min (1 hr from the beginning), the contracture was induced again in the same manner, and the peak tension of this contracture was regarded as the control (100%) value in each muscle preparation. This procedure was repeated 6 times every 1 hr for 7 hr. Immediately after each potassium contracture, the muscle was washed out.

* Address: 540 Higashiyanagicho, Nishi-Iru, Shichihon-Matsu, Itsutsuji-Dori, Kamigyo-Ku, Kyoto 602, Japan.
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Table I. Composition of original Krebs-Ringer solution

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>118.1</td>
</tr>
<tr>
<td>KCl</td>
<td>3.4</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.8</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.2</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>25.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>11.1</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>2.5</td>
</tr>
</tbody>
</table>

pH 7.4-7.5 at 42°C when saturated with carbogen (5% CO₂+95% O₂).

Modification of the composition of Krebs-Ringer solution prevented the shift of pH with modified solutions while the four types of gas were aerated for 7 hr as illustrated in Table II.

Table II. Modification of Krebs-Ringer solution for various gas aeration, and resultant pH

<table>
<thead>
<tr>
<th>Gas</th>
<th>Concentration of NaHCO₃ (mM)</th>
<th>Resultant pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% CO₂+95% O₂</td>
<td>25.0</td>
<td>7.4-7.5</td>
</tr>
<tr>
<td>20% CO₂+80% O₂</td>
<td>60.0</td>
<td>7.4</td>
</tr>
<tr>
<td>100% O₂</td>
<td>1.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Air</td>
<td>1.2</td>
<td>7.4-7.5</td>
</tr>
</tbody>
</table>

Concentrations of other components were the same as the original.

Fig. 1. Schematic diagram of experimental apparatus.

Three times with fresh Krebs-Ringer solution.

Four types of aeration gas; (1) carbogen (CO₂ 5% + O₂ 95%), (2) CO₂ 20% + O₂ 80%, (3) O₂ 100% and (4) air were bubbled respectively into the modified Krebs-Ringer solution (Table II) after the control contracture so as to evaluate the effect of carbon dioxide on preservation of contractile responses.

Modification of the composition of Krebs-Ringer solution prevented the shift of pH with modified solutions while the four types of gas were aerated for 7 hr as illustrated in Table II.
Results and discussion. According to the $Q_{10}$ law applied to a biological preparation, the metabolic level of animal tissues might be elevated around 2-fold higher than the normal level, if the temperature of tissues is 10°C higher than normal. When an animal carcass is kept at 35°C\cite{14,15} for only a few hours, the muscle tissues should become pale, soft, and exudative (PSE) due to the denaturation of both actomyosin and myoglobin of the tissues by accumulation of the resultant lactic acid derived from the accelerated anaerobic glycolysis in the tissues.

Fig. 2. Representative experimental records of 100 mM K+-contractures at 42°C. A: 20% CO$_2$+80% O$_2$. B: 100% O$_2$.

Fig. 3. Changes in relative peak tension of 100 mM K+-contractures during 7 hr at 42°C. •: 5% CO$_2$ +95% O$_2$. ○: 20% CO$_2$+80% O$_2$. □: 100% O$_2$. ■: Air.

Typical sequential patterns of the potassium contracture of the M. soleus aerated with two types of gas (A, CO$_2$ 20% + O$_2$ 80%; B, O$_2$ 100%) are illustrated in Fig. 2. There was a certain level of potentiation of potassium contracture in the gas mixture of A (CO$_2$ 20% + O$_2$ 80%) and a progressive decay in the gas of B (O$_2$ 100%).

Interestingly, the isolated rat M. soleus retained almost normal pattern of potassium contracture even under the hyperthermic condition (42°C) for as long as 7 hr when a sufficient amount of oxygen and carbon dioxide were bubbled into
the Krebs-Ringer solution (Fig. 3). This suggests normal intracellular Ca\(^{2+}\) regulation in the muscle tissues at such a hyperthermic temperature.

The changes in the pattern of potassium contracture illustrated in Fig. 3 might occur by an appropriate environmental gas formulation which reflect the maintenance of intracellular Ca\(^{2+}\) regulation. These observations suggest that the gas tension of oxygen and carbon dioxide particularly the carbon dioxide contributes to preserve normal physiological activities in the isolated skeletal muscles.

Since the results of the present study were obtained at 42°C, the normal pattern of potassium contracture could be maintained longer at a certain lower temperature according to the Q\(_{10}\) law.

Finally, this study provides evidence that an appropriate amount of carbon dioxide preserves animal tissues for a long period.

References
3) Graham, G. R. et al. (1959) : ibid., 149, 75–76.