Effect of Malonate, Succinate and Glucose, *In Vivo*, on the Uptake of Cesium-137 by Pigeon (*Columba livia*) Muscles

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ABSTRACT

The *in vivo* effects of malonate, succinate and glucose on $^{137}$Cs uptake by muscles of pigeon have been studied. At 15 $\mu$g malonate/g body the radionuclide uptake in red muscle, white muscle, cardiac muscle and smooth muscle was found to be reduced by 27\%, 14\%, 23\% and 27\% respectively in comparison to the controls. The inhibition of radiocesium uptake has been even more pronounced at 25 $\mu$g malonate/g body with as much as 68\% reduction of uptake in red muscle. Succinate and glucose enhanced the uptake of $^{137}$Cs in varying degrees in all the muscles. Moreover, both succinate and glucose could overcome the inhibitory effect of malonate in all the tissues except smooth muscle.

INTRODUCTION

Studies on the distribution of $^{137}$Cs in pigeon muscles have shown that 1 hour after injection there is a great disparity in the amount of the radionuclide present in the various muscles, the red muscle having nearly 4 times the radioactivity of that in the white muscle (Eapen and Narayanan13). Twenty four hours after injection, red muscle, white muscle and cardiac muscle have more or less similar radio-cesium burden (Eapen and Narayanan13). Cesium and potassium, both being alkali metals, are interrelated25 and under appropriate conditions one may displace the other in biological systems. In the light of the above report and that by Sreter and Woo31 that white muscle has a higher potassium content than the red muscle, the

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initially higher amount of cesium in red muscle when compared to white muscle is not readily understandable. Perhaps, the reason for the disparity lies in the characteristically different metabolism of the two types of muscle. The oxidative metabolism of red muscle is strikingly higher than that of white muscle. Sjodin and Beauge have shown that cesium transport into muscle cells is energy-dependent. It is likely, then, that the initially higher uptake of $^{137}$Cs by red muscle may have been influenced by its higher oxidative metabolism. Under the circumstances, extraneous inhibitors or substrates of oxidative metabolism would either inhibit or activate $^{137}$Cs uptake by muscle. To investigate this, the effects of malonate which is a specific inhibitor of succinic dehydrogenase, and of succinate and glucose on the in vivo uptake of $^{137}$Cs by muscles of pigeon have been studied.

**MATERIALS AND METHODS**

Pigeons (*Columba livia*) weighing 250-300 g were used for all the experiments. Four concentrations of malonate viz. 0.05 $\mu$g/g, 1 $\mu$g/g, 15 $\mu$g/g and 25 $\mu$g/g body and one, 50 $\mu$g/g body, of either glucose or succinate were used for injections in 0.1 ml of distilled water. The controls received the same amount of distilled water. Approximately 10 $\mu$Ci $^{137}$Cs was injected 30 min. after any one of the above injections, all injections being intramuscular. In a separate set of experiments 25 $\mu$g malonate/g body was injected 30 min. before consecutive injection of 50 $\mu$g succinate or glucose/g body and 10 $\mu$Ci $^{137}$Cs. All the birds were sacrificed 1 hour after the last injection viz. that of the radionuclide. Samples of *M. pectoralis* (red muscle), leg muscles (white muscle), heart (cardiac muscle) and gizzard (smooth muscle) were collected and digested in 15% NaOH. The aliquots, in duplicate, were plated on stainless steel planchets and counted with an end-window GM counter. The results were computed as "relative activity" i.e. $\frac{cpm/g \text{ tissue}}{cpm/g \text{ body weight (dose)}}$.

The effect of malonate on oxygen consumption of red muscle was measured in a manometric system. The birds were injected with 25 $\mu$g malonate/g body in 0.1 ml distilled water or with 0.1 ml distilled water alone. The birds were sacrificed 30 min. after the injection. A 10% homogenate of *M. pectoralis* was prepared in Ringer-phosphate buffer (pH 7.4). Three milliliter aliquots were transferred to the flasks, the central wells of which contained 0.2 ml of 10% KOH and a small roll of filter paper. The oxygen consumption was measured for 30 min., after 5 min. of equilibration, at 37°C in a Gilson differential respirometer. The results were computed as $\mu$l oxygen/100 mg fresh tissue.

**RESULTS**

Malonate did not show any effect on $^{137}$Cs uptake at concentration of 0.05 $\mu$g/g and 1 $\mu$g/g body. At 15 $\mu$g, the radionuclide levels of red muscle, white muscle, cardiac muscle and smooth muscle have been reduced by 27%, 14%, 23% and 27% respectively in comparison to the controls. The inhibition in radiocesium uptake has been even more pronounced at 25 $\mu$g malonate/g body with as much as 68%
Table 1. Effect of malonate, succinate and glucose on $^{137}$Cs uptake by pigeon muscles 1 hour after injection.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Malonate $^{15}$ μg/g body (a)</th>
<th>Malonate $^{25}$ μg/g body (a)</th>
<th>Succinate $^{50}$ μg/g body (a)</th>
<th>Glucose $^{50}$ μg/g body (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red muscle</td>
<td>0.99*</td>
<td>0.72</td>
<td>0.32</td>
<td>1.26</td>
<td>1.21</td>
</tr>
<tr>
<td>White muscle</td>
<td>0.56</td>
<td>0.48</td>
<td>0.34</td>
<td>1.12</td>
<td>0.77</td>
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<tr>
<td>Cardiac muscle</td>
<td>4.10</td>
<td>3.16</td>
<td>2.69</td>
<td>7.31</td>
<td>4.87</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>3.32</td>
<td>2.44</td>
<td>1.87</td>
<td>7.29</td>
<td>2.46</td>
</tr>
</tbody>
</table>

(a) injected 30 min. before $^{137}$Cs administration.
* Each figure is the average of 5 experiments.

Table 2. Effect of succinate and glucose on malonate-inhibition of $^{137}$Cs uptake by pigeon muscles.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Malonate $^{25}$ μg/g, succinate $^{50}$ μg/g body (a)</th>
<th>Malonate $^{25}$ μg/g, glucose $^{50}$ μg/g body (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red muscle</td>
<td>0.99*</td>
<td>1.34</td>
<td>1.07</td>
</tr>
<tr>
<td>White muscle</td>
<td>0.56</td>
<td>0.64</td>
<td>0.62</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>4.10</td>
<td>5.70</td>
<td>4.64</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>3.32</td>
<td>2.69</td>
<td>2.66</td>
</tr>
</tbody>
</table>

(a) Malonate injected 30 min. prior to consecutive injection of succinate and $^{137}$Cs.
(b) Malonate injected 30 min. before consecutive administration of glucose and $^{137}$Cs.
* Each figure is the average of 57 experiments.

reduction in the red muscle (Table 1). Succinate enhances the uptake of $^{137}$Cs in all the muscle and glucose in all except smooth muscle, the former showing the maximum effect on the smooth muscle and the latter on the white muscle (Table 1).

Succinate and glucose are capable of overcoming the inhibitory effect of malonate in all the tissues except the gizzard in which there is only partial reversal (Table 2). The oxygen consumption of red muscle from control and malonate treated pigeons is 39.2 μl O₂/100 mg and 29.8 μl O₂/100 mg fresh tissue respectively.

DISCUSSION

The translocation of ions across biological membranes is generally energy-dependent, the energy being quite often derived from oxidative metabolism. A nucleated mammalian erythrocytes which do not respire, however, depend upon glycolysis to provide energy for ion transport whereas nucleated red cells of reptiles and birds derive energy for the process through aerobic metabolism. There
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are systems, like the duck erythrocytes, where energy for ion transport is derived through either glycolysis or oxidative metabolism\(^{10}\). Sjodin and Beauge\(^{6}\) and Beauge and Sjodin\(^{11}\) find that translocation of cesium into frog sartorius muscle is strongly suppressed by strophanthidin and suggest that Cs\(^+\) uptake by muscle is dependent upon active metabolism. Malonate is known to inhibit ion transport which depends upon aerobic metabolism\(^{12}\). Results obtained in the present study show that malonate does inhibit \(^{137}\)Cs uptake by muscle. Since the levels of oxidative metabolism are different in the various types of muscle, presumably, the degree of inhibition of the radionuclide uptake by malonate is characteristically different. This is clearly seen in the red and white muscles where the inhibition is in direct relation to their aerobic metabolism. White muscle with its high glycolytic activity\(^{5}\) has not been as severely affected as red muscle. This is in conformity with observations on frog skeletal muscle (white muscle) in which ion transport has been shown to be unaffected by cyanide\(^{13}\). The pigeon white muscle, like the mammalian erythrocyte and frog skeletal muscle, may depend upon glycolytic metabolism for transport of ions like Cs\(^+\). The low but still appreciable inhibition in white muscle and lack of complete inhibition in red muscle is likely to be because both the muscles are not homogeneous. The white muscle has a certain proportion of red muscle fibres and *vice versa* in the red muscle. Moreover, neither are white muscle fibres dependent exclusively upon glycolysis nor do red muscle fibres rely entirely on oxidative metabolism.

Malonate is a competitive inhibitor of succinic dehydrogenase and addition of excess succinate would remove the inhibition caused by the former. This is apparent in all the muscle studied except the gizzard. However, the fact that glucose could do the same would indicate that it is also possible to have non-specific reversal. Succinate and glucose, when present by themselves, elevate \(^{137}\)Cs uptake by the muscles. However, succinate is the more efficient of the two in this respect.

From the foregoing discussion it may be surmised that \(^{137}\)Cs uptake by the red muscle and white muscles is influenced by the inherent differences in their metabolism. This is amply demonstrated by the muscles' responses towards added inhibitor and substrates. Cardiac and smooth muscles also show characteristic patterns of uptake of \(^{137}\)Cs.

REFERENCES