SHORT COMMUNICATION

SOME PROPERTIES OF RED BLOOD CELL GLUCOSE-6-PHOSPHATE
DEHYDROGENASE FROM THREE SPECIES OF KANGAROO*

By B. J. Richardson† and A. B. Czuppon‡

Richardson and Czuppon (1969) reported a cline in red blood cell glucose-6-
phosphate dehydrogenase levels in a marsupial—the wallaroo Macropus robustus
Gould, 1841. High dehydrogenase levels were found in animals from the dry inland
areas of Australia, while animals from the wetter eastern coastal areas had lower
levels. Richardson and Czuppon (1970) presented evidence to show that this
phenomenon was inherited and not the result of direct environmental influences.
The variation in dehydrogenase levels could be due to altered function of the enzyme,
variations in the rate of production of the enzyme, or to a change in the half-life of
the enzyme. This communication reports some biochemical properties of the
enzyme. These properties were also determined for enzyme preparations from two
related species—the grey kangaroo, Macropus giganteus Shaw, 1790, and the red
kangaroo, Megaleia rufa (Desmarest, 1822).

The methods used for purifying the enzyme and for determining $K_m$ values with
glucose 6-phosphate and TPN as substrate were those recommended by Kirkman (1962).
Utilization of the substrate analogue 2-deoxyglucose 6-phosphate relative to glucose
6-phosphate was determined for each animal. For the pH activity curves the standard
assay procedure (Zinkham, Lenhard, and Childs 1958) was used with the pH of the
Tris–HCl being altered to give the desired pH in the final solution.

The results are summarized in Tables 1 and 2. The purified enzyme preparation
contained less than 1% glucosephosphate isomerase and less than 0.01% 6-phosphogluconate dehydrogenase.

In the three species examined the $K_m$ values with glucose 6-phosphate and
TPN are markedly higher than those reported for humans (Kirkman 1962). While
the $K_m$ TPN values for the inland and coastal wallaroos are indistinguishable, a
slight difference is apparent between the $K_m$ glucose 6-phosphate values. This
variation, which would lead to a difference of about 1 unit of activity between
inland and coastal animals under the normal assay conditions, is insufficient to
explain the difference of 13 units found between the two forms.

The pH activity curves for the dehydrogenases of the inland and coastal
wallaroos and of the red kangaroo were similar. The grey kangaroo preparation
was active over an even wider range of pH and the curve differed in shape from
that of the other kangaroos, being biphasic.

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N.S.W. 2033.
While the properties of the glucose-6-phosphate dehydrogenase of these three species have much in common, as would be expected from their close phylogenetic relationship, in each case there is some slight, presumably evolutionary, variation. The reported variation in dehydrogenase levels between inland and coastal forms was not due to alteration in any of the properties studied.

**Table 1**

**SUMMARY OF THE BIOCHEMICAL PROPERTIES OF THE PURIFIED GLUCOSE-6-PHOSPHATE DEHYDROGENASE PREPARATIONS**

<table>
<thead>
<tr>
<th>Species*</th>
<th>Glucose-6-Phosphate Dehydrogenase Level in Population (µg/g Hb)†</th>
<th>K_m (µM)</th>
<th>Relative 2-Deoxyglucose 6-Phosphate Utilization</th>
<th>Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose 6-Phosphate</td>
<td>TPN</td>
<td></td>
</tr>
<tr>
<td><em>Macropus robustus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inland (2)</td>
<td>25.7±3.2 (21)</td>
<td>110, 125</td>
<td>37.5, 40</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Coastal (2)</td>
<td>13.9±1.5 (23)</td>
<td>147, 150</td>
<td>37, 37</td>
<td>&lt;1%</td>
</tr>
<tr>
<td><em>Megaleia rufa</em> (1)</td>
<td>4.65±0.4 (55)</td>
<td>140</td>
<td>15.5</td>
<td>&lt;1%</td>
</tr>
<tr>
<td><em>Macropus giganteus</em> (1)</td>
<td>25.5±3.7 (8)</td>
<td>125</td>
<td>37</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Human*</td>
<td>4.22±0.11 (202)</td>
<td>50-78</td>
<td>2.9-4.4</td>
<td>&lt;4%</td>
</tr>
</tbody>
</table>

* No. of preparations given in parenthesis.
† Mean values ±95% confidence limits given. No. of determinations given in parenthesis.

**Table 2**

**ACTIVITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE AT VARIOUS pH VALUES**

Activities expressed as a percentage of the activity at pH 9.0. Number of preparations (n) given in parenthesis. When n was > 1, the average was taken.

<table>
<thead>
<tr>
<th>Species</th>
<th>pH</th>
<th>3</th>
<th>6.5</th>
<th>7.05</th>
<th>7.6</th>
<th>8.0</th>
<th>8.4</th>
<th>9.0</th>
<th>9.5</th>
<th>10.4</th>
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</thead>
<tbody>
<tr>
<td><em>Macropus robustus</em></td>
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<td></td>
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</tr>
<tr>
<td>Inland (n=2)</td>
<td></td>
<td>0</td>
<td>39</td>
<td>61</td>
<td>78</td>
<td>93</td>
<td>97</td>
<td>100</td>
<td>82</td>
<td>5</td>
</tr>
<tr>
<td>Coastal (n=1)</td>
<td></td>
<td>0</td>
<td>29</td>
<td>50</td>
<td>83</td>
<td>94</td>
<td>97</td>
<td>100</td>
<td>69</td>
<td>0</td>
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<tr>
<td><em>Megaleia rufa</em> (n=1)</td>
<td></td>
<td>5</td>
<td>35</td>
<td>60</td>
<td>85</td>
<td>95</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>15</td>
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<tr>
<td><em>Macropus giganteus</em> (n=3)</td>
<td></td>
<td>27</td>
<td>54</td>
<td>79</td>
<td>92</td>
<td>83</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>6</td>
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<tr>
<td>Human*</td>
<td></td>
<td>63</td>
<td>72</td>
<td>84</td>
<td>91</td>
<td>96</td>
<td>100</td>
<td>87</td>
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</table>

* From Kirkman (1962).

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**References**