ASSOCIATION BETWEEN SERUM ALKALINE PHOSPHATASE VARIANTS
AND THE R-O-i BLOOD GROUP SYSTEM IN THE AUSTRALIAN MERINO*

By R. M. Hope†

Rendel and Stormont (1964) reported evidence of a polymorphism in sheep for
serum alkaline phosphatase. Starch gel electrophoresis of serum always revealed
a single zone of enzyme activity (zone A) on the cathode side of the transferrins.
In some samples, however, an additional zone of slower mobility (zone B) was
observed closer to the slow α-globulins, and the presence of this zone was closely
associated with the presence of soluble blood group substance O in the plasma and
on the red cells. Associations have also been described between electrophoretically
distinguishable serum phosphatase types and the J blood group system of cattle
(Rendel and Gahne 1963), and the ABO blood group system of man (Beckman
1964). The blood groups concerned with these associations (R-O-i of sheep, J of
cattle, and ABO of man) are serologically closely related (Neimann-Sorensen, Rendel,
and Stone 1954), and the finding of similar associations with related blood groups in
three organisms seems to strengthen the possibility of some general functional
relationship between alkaline phosphatase and blood groups. However, the nature
of the genetic control of serum alkaline phosphatase is not known, and it is possible
that the difference in phosphatase phenotype is due to interaction between the
enzyme and certain blood group substances. This suggestion is supported by the
artificial induction of B zone alkaline phosphatase activity in the serum of previously
B-negative sheep by intravenous injection of saliva containing O blood group
substance (Rendel et al. 1964).

Rendel et al. (1964) found sheep family data difficult to interpret, as sera from
young lambs, regardless of blood group, revealed B zone activity. Rasmussen (1965)
confirmed from family data the association between B phosphatase activity and
O blood group substance in several British breeds of sheep. The purpose of this
communication is to report data for the Australian Merino showing the association
first described by Rendel and Stormont (1964), and also to report some family data
for the alkaline phosphatase variants, and the R-O-i blood groups.

Serum samples were obtained from 117 Merino sheep of known pedigree and
R-O-i blood group. These sheep were chosen from an experimental flock maintained
by the Department of Agriculture at Kybybolite, South Australia. Starch gel
electrophoresis was carried out as described by Rendel and Stormont (1964). The
staining solution consisted of 100 ml distilled water, 100 mg garnet GBC salt, 100 mg
sodium α-naphthyl phosphate, and 10 drops of 10% magnesium sulphate.

Sera from all animals revealed an A zone. Animals were classified into two
groups corresponding with the presence (+) or absence (−) of the B zone. This

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classification differs from that used by other authors (Rendel and Stormont 1964; Rasmusen 1965) who prefer to classify into more than two groups depending on an arbitrary scale of $B$ zone intensity. In this material considerable variation was noted in the staining intensity of the $B$ zone between samples. However, variation was also noted in replicate runs of the same sample so that considerable error may be involved in such a classification based on intensity. A sample showing very weak $B$ zone activity was run on each gel as an indicator of such variation but some misclassification of weak positives may still have occurred.

It can be seen from Table 1 that in this material a marked association exists between $R-O-i$ blood group and phosphatase classification, the sera from group $R$ animals rarely showing $B$ zone activity. Each of the five group $R$ sheep recorded as showing $B$ zone activity had a comparatively weak staining $B$ zone.

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Phosphatase Classification</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Sheep with $B$ Zone Present</td>
<td>No. of Sheep with $B$ Zone Absent</td>
</tr>
<tr>
<td>$R$</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>$O$</td>
<td>61</td>
<td>11</td>
</tr>
<tr>
<td>Totals</td>
<td>66</td>
<td>51</td>
</tr>
</tbody>
</table>

* These animals are parents and offspring shown in family data (Table 2).

It can be seen from Table 2 that the family data agree with expectations based on the postulated genotypes for the $R-O-i$ blood group phenotypes, group $O$ being due to a homozygous recessive condition at an autosomal locus (Rendel et al. 1964). (No sheep of blood group $i$ was found.) Three rams were involved in the matings shown, two being of group $R$ and one of group $O$. The two group $R$ rams were presumably heterozygous, as group $O$ lambs resulted from their matings with group $R$ ewes. As recorded by Rasmusen (1965) the $O$ lambs from $R \times R$ matings showed $B$ zone activity, although the numbers are small. However, these results differ from those of Rasmusen who failed to detect $B$ zone activity in any of the $R$ lambs resulting from $R \times O$ crosses, whereas some such progeny were detected in the present series.

The origin of $B$ zone alkaline phosphatase remains unclear. One hypothesis (Rendel et al. 1964) postulates that the $B$ zone is the product of an interaction between a blood group substance present in sheep of blood group $O$ and an alkaline phosphatase component normally present in sera (possibly represented by the $A$ zone). The unusual phenotypes $R(\pm)$ and $O(-)$ would seem to constitute exceptions to this hypothesis. However, the $O(-)$ but not $R(\pm)$ phenotype may result from the
errors of classification noted above. Also the three $R(+)\text{ animals may have in fact been heterozygous at the blood group locus. A critical examination of the possible genetical control of these aberrant phenotypes must await more results of the testing of progeny of such } R(+)\text{ and } O(-)\text{ animals. The seven progeny with the rare combinations of phosphatase type and blood group do not seem to fit any consistent pattern of parental mating type.}

The in vivo function of serum alkaline phosphatase is unknown. Rendel et al. (1964) have shown that sheep of blood group O have on the average a 75% higher serum alkaline phosphatase activity than sheep of blood group $R$, this difference presumably being due to the additional phosphatase represented by the $B$ zone in the majority of such animals. It would seem that effective selection for increased serum alkaline phosphatase, should this prove desirable, could be made through the $R-O-i$ blood group system.

I would like to thank the Department of Agriculture at Kybybolite, for making available the serum used in this survey, and Dr. D. W. Cooper for determination of the blood groups. I am very grateful to Professor J. H. Bennett and Dr. M. J. Mayo for their constant help and encouragement in this work.

<table>
<thead>
<tr>
<th>Female Parents × Male Parents</th>
<th>No. of Matings</th>
<th>No. of Progeny of Blood Group $R$ ($R/r$)</th>
<th>No. of Progeny of Blood Group $O$ ($r/r$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R-(-)\ddagger × Br(-)$</td>
<td>4</td>
<td>1§</td>
<td>3§</td>
</tr>
<tr>
<td>$Br(-) × Br(-)$</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R(-+) × Br(-)$</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Br(+) × Br(-)$</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$rr(-) × Br(-)$</td>
<td>5</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>$rr(+) × Br(-)$</td>
<td>20</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>$R(-) × rr(+)</td>
<td>6</td>
<td>1§</td>
<td>7§</td>
</tr>
<tr>
<td>$Br(-) × rr(+)</td>
<td>3</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>$R(-+) × rr(+)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Br(+) × rr(+)</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$rr(+) × rr(+)</td>
<td>8</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>$rr(-) × rr(+)</td>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>51</td>
<td>3</td>
<td>24</td>
</tr>
</tbody>
</table>

* Blood group genotypes have been inferred from phenotypes of parents and their offspring.
† $(+)$ denotes serum showing $B$ zone alkaline phosphatase activity; $(-)$ denotes serum showing no detectable $B$ zone activity.
‡ $R-$ signifies genotype could be $R/R$ or $R/r$.
§ Genotypes could be $R/R$ or $R/r$. 

\[\text{Table 2}\]

\[\text{Family data showing serum phosphatase classifications and } R-O\text{ blood groups*}\]
References


