INHERITANCE OF R-O-i BLOOD GROUPS AND ALKALINE PHOSPHATASE POLYMORPHISM IN SHEEP¹

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RENDEL and STORMONT (1964) found that in sheep sera subjected to starch-gel electrophoresis the zone of phosphatase activity which they designated B is highly correlated with the presence of soluble blood-group substance O in adult females. Since the presence of O substance is recessive to the presence of R substance (STORMONT 1951; RENDEL, NEIMANN-SØRENSEN and IRWIN 1954), they inferred that the presence of the B zone was also a recessive trait. RENDEL, AALUND, FREEDLAND and MØLLER (1964), in tests of yearling sheep, confirmed the association between B phosphatase activity and O substance. However, tests of sera of lambs 2½ to 4 months of age revealed a less marked association; there was some B phosphatase in the serum of most lambs regardless of their R-O blood groups, although the mean B-zone score in lambs of group O was significantly greater than in lambs of group R.

The data presented in this report were collected as part of a survey of serum alkaline phosphatase types of sheep and pigs. Tests of sera of sheep of groups R, O and i provide additional data on the association between the B phosphatase zone and group O in sheep and the absence of the B zone in R and i sheep. The A-O blood-group system in pigs (RASMUSEN 1964) is related to the R-O system in sheep, but no polymorphism of alkaline phosphatase types in pigs related to A-O blood groups was found when sera from pigs were tested using the same techniques as for sheep. Sera from Duroc and Yorkshire pigs all had a phosphatase band comparable to the A band of sheep, and all sera tested lacked a band comparable to the B band, regardless of whether they were of group A, O or "—". Therefore only the data for sheep are reported here.

MATERIALS AND METHODS

Blood samples were obtained from 307 sheep. These included 75 Rambouillets (35 lambs 4½ to 8½ months old and 40 adults) bled in 1961, 25 Shropshires (14 lambs 5 to 7 months old, 2 yearlings and 9 adults) bled in 1961, 17 Shropshires (16 lambs 4½ to 6 months old and 1 adult) bled in 1962, 44 Southdowns (15 lambs 4 to 5 months old, 4 yearlings and 25 adults) bled in 1962, 62 Southdowns including 14 from the 1962 bleeding (24 lambs 3 to 4 months old, 10 yearlings and 28 adults) bled in 1964, and 98 crossbred yearling females of undetermined ancestry bled in 1961. The purebred sheep were of known ancestry, so that data from families were useful to study the inheritance of R-O-i blood groups and the B phosphatase zone. Red cells were tested in a standard hemolytic test to determine R-O-i phenotypes, soon after the samples were collected. Serum samples were frozen and kept at -20°C. All sera were tested for alkaline phosphatase

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types in 1964; those which had been stored for 2 or 3 years were typed as readily as those collected in 1964.

The alkaline phosphatase types were determined with minor modifications of the technique described by RENDEL and STORMONT (1964). Filter papers soaked in the serum samples were inserted 2 cm from the edge of the gel parallel to the long axis, and filter paper wicks from the buffer solution on the cathode side of the gel were overlapped onto 1 cm of the gel. A cold room (5 to 8°C) was used for electrophoresis. The sliced half of the gel was incubated in the staining solution at room temperature overnight before phosphatase zones were classified. The B phosphatase zone for each tested serum was compared with that of a standard sample from a group-O sheep included on all gels. The intensity of the B zone was classified by two observers on an arbitrary scale as + (4), ± (3), tr (2), ? (1) and negative or 0. The ? classification was used when there was a faint indication of a B zone which was considered to be of doubtful significance. Serum samples classified as ? were retested, as were sera from group-O sheep which were found to have been classified as negative and sera from R or i sheep classified as positive for the B zone.

RESULTS AND DISCUSSION

The classification of the B zone of alkaline phosphatase is given in Table 1. Intermediate classifications are averages for samples which when retested were placed in a different class from the original test. The presence of the B zone in sera from lambs, yearlings and adults was clearly associated with blood group O in all breeds tested. Most sera from group-O sheep could be readily identified on the basis of the presence of a definite B zone, although in older ewes there were a number of cases where the B band was classified as ? or 0. These sera may be comparable to those of RENDEL *et al.* (1964) in which they demonstrated a B zone when they concentrated the sera.

The results given in Table 1 indicate a smaller proportion of group-R lambs with more than a faint indication of a B zone than RENDEL *et al.* (1964) observed. The lambs were, in part, somewhat older than theirs (see MATERIALS AND METHODS), but of the 1964 Southdown lambs which were not over 4 months old, only one of 12 R and two of eight i lambs had a ? B zone and the others were negative. All of the lambs had been weaned before bleeding, but this would not

TABLE 1
The B zone of alkaline phosphatase in sera of sheep of various ages

Age	Blood group	Total number	Classification of B zone									Average score
			+	±	tr	?	0	?	0	?	0	
			(4.0)	(3.5)	(3.0)	(2.5)	(2.0)	(1.5)	(1.0)	(0.5)	(0.0)	
Lambs	R	52	0	0	1	0	1	0	7	0	39	0.27
Lambs	O	42	13	1	14	0	8	2	1	1	2	2.81
Lambs	i	10	0	0	0	0	0	0	2	1	7	0.25
Yearlings	R	67	0	0	1	0	3	1	5	0	57	0.23
Yearlings	O	38	10	0	17	0	5	3	3	0	0	2.86
Yearlings	i	9	0	0	0	0	0	0	1	0	8	0.11
Adults	R	52	0	0	0	1	0	1	2	5	43	0.16
Adults	O	30	6	2	4	0	5	2	1	7	3	2.02
Adults	i	7	0	0	0	0	0	2	1	0	4	0.57

seem to be the reason for the lack of B phosphatase. Even though sheep milk from ewes of all R-O-i groups contains B phosphatase, only a very early transfer of serum phosphatases from the milk of the dam to the lamb would be expected (RENDEL *et al.*, 1964). Possibly other environmental factors as well as genetic background may influence the amount of B phosphatase in the serum, and differences in technique may also have influenced the results.

The R-O blood groups and average scores for the phosphatase B zones for lambs from various matings are given in Table 2. These data are in accord with those of RENDEL *et al.* (1964) who found that group-R lambs heterozygous for the *r*^o allele (all of those from matings of R × O) do not have more intense B-phosphatase zones than R lambs from R × R matings which presumably include some lambs which are homozygous for the *R* allele.

Two Southdown rams were i, and although they had too few offspring to draw definite conclusions concerning the possible effect of heterozygosity for the *I* gene (which is necessary for the expression of R and O) on expression of the phosphatase B zone, it is obvious that the *II* genotype is not necessary for the presence of the B zone. The three group-O lambs with an i sire had B-zone scores of 0, 1.5 and 4.

The B zone was definitely present in all of the seven group-O lambs from R × R matings and in none of their group-R parents as expected from the known recessive inheritance of group-O and the close association of group O and the presence of the B zone. The six i lambs, one from an i × O mating and five from i × i, all were negative for the B zone, as expected if O substance is necessary for the presence of the B phosphatase zone.

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SUMMARY

R-O-i blood groups and alkaline phosphatase types were determined for 307 sheep, including 65 lambs and their parents. As reported by other workers, a high degree of association was found between the presence of the B zone of alkaline phosphatase and group O, and the absence of the B zone and groups R and i. Family data confirm these associations.

TABLE 2
The B zone of alkaline phosphatase in lambs from parents of different R-O-i types

Parents		R lambs		O lambs		i lambs	
Male	Female	Total number of lambs	Average B-zone score	Total number	Average B-zone score	Total number	Average B-zone score
R × R		33	26	7	3.43	0	...
R × O		12	5	7	3.14	0	...
O × O		6	0	6	2.67	0	...
i × R		5	4	1	1.50	0	...
i × O		4	1	2	2.00	1	0.00
i × i		5	0	0	...	5	0.00

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