ANALYSIS OF SHEEP HEMOGLOBINS BY ISOELECTRIC
FOCUSING IN POLYACRYLAMIDE-GEL

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A new technique of isoelectric focusing in polyacrylamide-gel (PAG-IEF) was used to separate hemoglobins (Hb) from hemolysates of sheep red cells, the results of which were compared with the zymogram obtained by starch-gel electrophoresis (SGE). The isoelectric point (pI) of the Hb was measured and genetic system in Hb types was also examined.

As a result of PAG-IEF, the sheep Hb patterns were divided into 5 types, i.e., Hb1, Hb2, Hb2-1, Hb1' and Hb2-1'. The types Hb1, Hb2 and Hb2-1 were composed of 7, 8 and 13 bands, respectively. The types Hb1' and Hb2-1' were distinguished by the presence or absence of a band that indicated pI 6.93 in Hb1 and Hb2-1. The types Hb1 and Hb1' were corresponded to type HbB by SGE, type Hb2, to type HbA and types Hb2-1 and Hb2-1' to type HbAB, respectively. The bands of the type Hb1 appeared in a

Table 1. Conditions of isoelectric focusing in polyacrylamide gel.

- a) The gel is prepared as follows:
5.5% acrylamide-bisacrylamide solution	11.5ml
Saccharose	1.8g
Ampholine(pH6-8 range, 40%w/v, LKB)	0.9ml
Riboflavin 10mg%	0.2ml
- b) Electrode solution
Anode	1% acetic acid
Cathode	1% ethylenediamine
- c) Electrophoresis is performed as follows:
- The starting voltage is adjusted at 600volts, the voltage being increased to a maximum of 1,000volts. Final focusing occurs after from 4 to 6 hours.
- d) Gel size
- 170 x 140 x 0.5mm

Fig. 1. Electrophoretograms of sheep hemoglobins by isoelectric focusing in polyacrylamide-gel (A) and starch-gel electrophoresis (B).

Table 2. Inheritance data for hemoglobin types of the sheep.

Mating type	No. of mating	No. of offspring			Total	X-test
		1	2-1	2		
1 x 1	12	15			15	-
1 x 2-1	6	4	3		7	0.8 P 0.7
2-1 x 2-1	6	2	3	1	6	0.9 P 0.8
2 x 1	5		6		6	-
2 x 2	4			4	4	-

Table 3. Frequencies of hemoglobin types and their gene frequencies in the different breeds of sheen and test for Hardy-Weinbreg equilibrium.

Breed	Hb		Observed value			Expected value		
	Type	No.	%	Gene frequency		No.	%	X-test
Corriedale	1	54	72.97	Hb'	0.838	51.97	70.23	0.0380
	2-1	16	21.63	Hb	0.162	20.10	27.16	1.0130
	2	4	5.41			1.93	2.61	0.7260
Border Leicester	1	10	83.33	Hb'	0.917	10.09	84.08	0.0004
	2-1	2	16.67	Hb	0.083	1.83	15.26	0.0076
	2	0	0.00			0.08	0.67	0.0800
Suffolk	1	14	87.50	Hb'	0.938	14.08	88.00	0.0002
	2-1	2	12.50	Hb	0.062	1.86	11.63	0.0050
	2	0	0.00			0.06	0.37	0.0600
Romny Masgh	1	23	74.19	Hb'	0.855	22.66	73.10	0.0026
	2-1	7	22.58	Hb	0.145	7.69	24.81	0.0324
	2	1	3.23			0.65	2.09	0.0742

gel range from pH 7.76 to pH 7.10, and the bands of the type Hb2, from pH7.43 to pH6.88.

The family data suggested the major Hb types newly classified as Hb1, Hb2 and Hb2-1 to be controlled by co-dominant alleles of Hb and Hb . This mode of inheritance proved the same that of Hb types had been classified by SGE.