Heredity (1973), **31** (3), 325-337

THE EFFECTS OF *Ka* AND *Hb* GENOTYPES ON BLOOD ELECTROLYTES AND HAEMOGLOBIN IN SHEEP

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Received 6.xii.72

SUMMARY

Potassium and sodium concentrations and their ratios, and Mean Corpuscular Haemoglobin Concentrations were estimated in the blood of 469 sheep of three breeds and their crosses. Some breed differences were statistically significant but cross breed values were like the means of the parental breeds.

Estimates for the effects of the genotypes *Ka* (potassium level) and *Hb* (haemoglobin type), breeding, age, sex and management, and sample batch were isolated. The haemoglobin genotypes influenced the MCHC through variation in packed cell volume. The potassium genotypes had no such effect. After adjustment the estimates for red cell sodium were shown to be complementary to estimates for red cell potassium.

1. INTRODUCTION

BALANCED polymorphism of haemoglobin type (locus symbol *Hb*) and of red cell potassium concentration (*Ka*) is widely exhibited in sheep. Apart from a statistical association reported in some but not all populations, the presence of a causal relationship between the phenotypes has been suggested (see Agar *et al.*, 1972 for references). This is uncertain because other factors modify their expression to an obscuring and largely unknown degree.

It is important to isolate the primary effect of these genotypes and to understand the nature of any interaction. Both potassium ion and haemoglobin molecule are integral parts of red cell structure and function. Any change in either must have primary physical effects on the cell. The chain of subsequent events maintaining physiological homeostasis has not been described. It may be short and insignificant, or it may involve other mechanisms. It may even result in a modification of the individual's response to environmental pressures on a biological scale, or to production pressures on an agricultural scale. Claims for associations of blood types with commercially useful qualities abound, but in none is the connection known to be a chain of molecular events.

In this paper the effects of factors believed to influence the expression of the genes at these loci are investigated by analysis of laboratory data from an experimental flock. Each has its interest but estimates of the primary quantitative effects of the *Ka* and *Hb* genotypes are isolated; the relations with sodium are determined; interactions and secondary effects are explored; and data are given against which possible consequences for the red cell can be tested.

2. Materials and methods

The sheep used belonged to an experimental flock (the CIB) in which three breeds, Scottish Blackface Mountain, South Country Cheviot and Welsh Mountain were being compared with each other and with crosses made among them. At the stage of the experiment from which these findings are reported the flock was composed of pure bred animals and " crossbred " animals (table 1). The " crossbreds " theoretically had half of their genetic component from one breed and half from the other. They were generated in several ways not distinguished here (Wiener, 1967).

Table 1

Sheep samples classified by breed

				Total
Purebred	Blackface	61		
	Cheviot	50		
	Welsh	50	161	
" Crossbred "	Blackface × Cheviot	98		
	Blackface × Welsh	115		
	Cheviot × Welsh	95	308	469

" Crossbred " animals were generated in various ways; see text.

The numbers of sheep in the six breed classes are given in table 1; the age and sex distribution at the time of blood sampling and proximity to lambing in table 2; and the numbers of sheep classified by the *Hb* and *Ka*

Table 2

Sheep classified by sex, age at sampling and stage of pregnancy

				Stage of pregnancy					
				Number of days between sampling and lambing				Not mated or	
Age of class	Age in days	Sex: Males	Sex: Females	4-30	31-60	61-90	>90	barren	Total
1	358- 434	1	137	0	0	0	0	137	138
2	591- 715	38	73	18	27	23	1	4	111
3	934-1095	40	77	25	32	16	0	4	117
4	1320-1453	2	50	5	28	11	1	5	52
5	1715-1807	0	51	12	7	25	0	7	51
Totals		81	388	60	94	75	2	157	469

genotypes are in table 3. The distinction of heterozygous *Ll* from presumed homozygous *L* was made on the results of family studies (Eagleton *et al.*, 1970). Only about a quarter of the Hardy-Weinberg expectation of heterozygotes was detected, so that this distinction is presumed to be not fully disclosed.

(a) *Farm environment and management*

The upland farm Blythbank in Peeblesshire was of fields of sown pastures at 300 ± 30 metres above sea level. The sheep lived out of doors all the year round. They were separated by sex but not by breed, age, type of offspring nor rearing (*e.g.* twin or single). Females of age class 1 were not mated.

No females were blood sampled after lambing, and only seven lambed within a week of sampling. The ewes got no hay but had bruised oats and concentrate (" ewe nuts ") from 7th February to 11th May. Minerals were given *ad libitum* from 31st December to 2 weeks before lambing. Magnesium and

TABLE 3

Sheep sample classified by haemoglobin type (Hb) and potassium type (Ka)

	Red cell potassium concentration			
Phenotypically	Low	Low	High	Totals
Genotypically	*Ka L*	*Ka Ll*	*Ka ll*	
Hb AA	57	16	36	109
Hb AB	101	28	83	212
Hb BB	89	23	36	148
Totals	247	67	155	469

vitamin supplements were given to mid-June. The males' diet was supplemented by silage from mid-December to 6th February, then by rape and turnip residue to 6th March; salt licks were not provided. There was no veterinary or other evidence that these different managements had differing results.

(b) *Technique*

The sheep were regularly handled for weighing throughout the year. They were bled at the same time each Monday by the same people in 21 batches from December 1961 to June 1962. The composition of each batch was not random but followed management convenience. This uniformity may have reduced the emotional stress on the animals. An open needle was slipped into the jugular vein, the first few ml. was ignored, then 5 to 6 ml. were taken to a borosilicate glass tube containing 750 International Units of heparin*. Within 7 hours of bleeding, the packed cell volume was estimated by microhaematocrit† at 17,000 *g* for 10 minutes to minimise the trapped plasma content; and two dilutions of 0·25 ml. in 50 ml. were made, the first of whole blood and the second of plasma separated by centrifugation at 1000 *g* for 20 minutes. The correlation of adjusted estimates of cell and plasma potassium was subsequently calculated as $r = -0{\cdot}005$ showing that cell integrity was maintained through these procedures.

(c) *Estimations of potassium and sodium*

Two stock aqueous solutions of KCl‡ and NaCl‡ of 0·477 and 0·634 g./l. respectively were made and kept in polythene bottles. They sufficed for this work and that of Eagleton *et al.* (1970). From these daily working standards of 5 and 10 mg./l. K and Na were made. The flame photometer§ was standardised at zero for distilled water and the midscale point for the standard solution before and after every 8th or 12th sample.

The red cell estimates (per litre of cells) were calculated from the formula $[100\ (WB - Pl) \div PCV] + Pl$

* Evans Medical Ltd., Speke, Liverpool, U.K.
† Hawksley & Sons Ltd., Lancing, Sussex, U.K.
‡ " Specpure " by Johnson Matthey & Co., Ltd., London, U.K.
§ Evans Electroselenium Ltd., Halstead, Essex, U.K.

where WB = whole blood value in milli-equivalents per litre (= mEq./l.).
Pl = the same for plasma
PCV = packed cell volume per centum.

Other abbreviations are:

K_c = concentration of potassium in red cells
K_p = concentration of potassium in plasma
Na_c = concentration of sodium in red cells
Na_p = concentration of sodium in plasma

At the end of the estimations calibration curves were prepared by making 10 dilutions at equal intervals of each standard stock solution to cover the range 0 to 50 mEq./l. of potassium and 0 to 200 mEq./l. of sodium. The diluted standards were estimated in two ways, alone and mixed. The mixtures were in two sequences, one with low K mixed with low Na, through to high K mixed with high Na; and the other with low K mixed with high Na through to high K mixed with low Na. All these solutions were put through the flame photometer and the meter readings plotted against concentrations. There were very small differences between the expected and observed readings for K. The differences for Na were noticeable, the observed readings being higher than expected at the quarter scale point and lower at full scale. The presence or absence of either ion could not be seen to affect the estimate of the one under assay. Duthie and McDonald (1960) gave the magnitude of the interference effect of potassium on sodium as 1 per cent. for sheep whole blood and ½ per cent. for sheep plasma; and of sodium on potassium as 1½ per cent. and 2 per cent. respectively. These corrections were not used here. The scale readings were calibrated numerically using a local quadratic function. The calculations were done by a computer program subroutine.

(d) *Haemoglobin typing*

The red cell deposit from the tube centrifuged for plasma was washed twice with 10 volumes of 0·9 per cent. NaCl then lysed with 3 to 4 times its volume of distilled water. The solution was made up to an appropriate density as judged by eye and experience. Electrophoresis was carried out in boric acid—borate buffer *p*H 8·6, EMF of 6 volts per centimetre for about 6 hours on Whatman's No. 54 paper held horizontal in a Krogh* tank. The paper was then stained in 2 per cent. light green in 3 per cent. trichloracetic acid in water, washed to a white background in 4 per cent. glacial acetic acid in water, dried and stored.

(e) *Haemoglobin estimation*

The alkaline haematin method using the Gibson Harrison artificial standard† was used. The technique was as recommended by the makers of the standard, timing was by stopwatch and within 1 per cent. of that recommended. Room temperature was 19° ± 2° C. and corrections were not made for this slight fluctuation. The EEL‡ portable colorimeter with the 624 filter was used to estimate the optical densities.

* Messrs Shandon Scientific Co. Ltd., London, U.K.
† The British Drug Houses Ltd., Poole, U.K.
‡ Evans Electroselenium Company, Halstead, Essex, U.K.

(f) *Statistical analysis*

The data were analysed using a computer program especially designed to provide a least squares analysis of non-orthogonal data. This program is similar in aim to that described by Lewis (1968). The program estimates the effects of classifications (in this case breed, *Ka*, etc.) as regressions on real or dummy variates. Interactions can be estimated as well as main effects. Tables of fitted values are then constructed using the regression coefficients and the vectors of means of the dependent and independent variates. The program provides, in full, estimated variances of regressions and fitted values, and an analysis of variance. The analysis separates the sum of squares into two parts viz. a part due to regression and a part due to error. Unfortunately, it is not practicable to present the complete computer output here. The non-orthogonal structure of the data strictly requires a different standard error for each contrast of the table values, and probability statements in the discussion depend on these. However, we suggest that root mean square standard errors (based on the standard errors of the normalised contrasts between two expected values) are adequate for purposes of exposition. These average standard errors are termed " AV " S.E. and are presented with the tables of results. Also presented (where appropriate) are the largest and smallest standard errors appropriate to any contrast. These are denoted " MAX " S.E. and " MIN " S.E. respectively.

3. Results

Main effects were fitted for the effect of *Ka*, *Hb*, breed, age, sex and management and batch of samples. Two factor interactions were also tested but none was found to be significant at the 5 per cent. probability level. The numerical results are presented in the form of tables of fitted values (tables 4-9) for each classification, " averaged " over all other classifications.

There are six independently presented variates, namely estimates of potassium and sodium for red cells and plasma (variates 1, 2, 3, 4), the packed cell volume (PCV) (variate 12), and the haemoglobin concentration in grams per 100 ml. whole blood (variate 13). The mean corpuscular haemoglobin concentration (MCHC) is derived from the last two.

The other seven variates represent different relationships of the concentration of the two cations. The variances of the independent variates differed, and the ratios derived from them are presented in the form with the lower variance rather than its inverse. All derived variates tabulated were calculated on an individual basis (*i.e.* variates 5, 6, 7, 8, 9, 10, 11, 14).

The relationship of intracellular and extracellular potassium and sodium are given separately as the sum (9 and 10 respectively) and the ratio (5 and 6 respectively). The ratios of concentration between inside and outside the cell are given separately for potassium (the afferent process, variate 7); and sodium (the efferent process, variate 8).

Scatter diagrams were made and individuals were seen to be distinctly high or low potassium (abbreviated to HK and LK respectively) but the sodium values overlapped. Secondly, within each of these populations there was no obvious correlation between the concentrations of potassium and sodium in the red cells. The coefficients were $r = -0{\cdot}09$ and $r = -0{\cdot}28$ in the low and high respectively.

4. Discussion

The tabulated results show that nearly all the classifying factors had some influence on the red blood cells and their electrolytes and on the electrolytes of the plasma. The two main results are that the packed cell volume was altered by breeding, age, batch and *Hb* type but not by *Ka* type: and in general the sodium and potassium estimates were affected by breeding, batch and age, and of course, the *Ka* types, but also by the *Hb* types. Sex and management had no significant effects, but this statement is qualified.

(a) *The effect of the potassium genotypes* (*Ka*) (table 4)

First, the conclusion can be drawn that the PCV, haemoglobin concentration and MCHC were quite unaffected by the *Ka* genotype. Second, the

Table 4

Blood values for potassium concentration genotypes, adjusted for other effects

	Variate	Mean values			Standard errors		
		L	*Ll*	*ll*	"AV" S.E.*	"MAX" S.E.*	"MIN" S.E.*
1.	K_c	19·38	21·89	72·94	0·437	0·511	0·347
2.	Na_c	71·25	69·71	21·59	1·509	1·765	1·199
3.	K_p	5·018	5·281	5·699	0·0716	0·0837	0·0569
4.	Na_p	136·27	136·41	136·01	0·508	0·594	0·404
5.	Na_c/K_c	3·988	3·221	0·387	0·1092	0·1288	0·0868
6.	Na_p/K_p	28·11	26·56	24·48	0·497	0·581	0·395
7.	K_c/K_p	4·03	4·26	13·31	0·164	0·192	0·131
8.	Na_c/Na_p	0·527	0·512	0·162	0·0119	0·0140	0·0095
9.	Na_c+K_c	90·64	91·60	94·53	1·484	1·735	1·179
10.	Na_p+K_p	141·28	141·69	141·70	0·513	0·600	0·408
11.	$\frac{Na_c+K_c}{Na_p+K_p}$	0·6454	0·6482	0·6708	0·01198	0·01401	0·00952
12.	PCV %	35·341	35·178	35·412	0·2767	0·3237	0·2199
13.	Hb %	13·729	13·635	13·771	0·0573	0·0671	0·0456
14.	MCHC	39·04	38·98	39·15	0·227	0·265	0·180

Variates 1, 2, 3, 4, 9 10 in mEq./l.; 13 in grams haemoglobin per 100 ml. whole blood.

* See text.

K_c = Red cell potassium. Na_c = Red cell sodium. K_p = Plasma potassium. Na_p = Plasma sodium. PCV = Haematocrit. Hb = Haemoglobin. MCHC = Mean corpuscular haemoglobin concentration.

mean (adjusted) values for red cell sodium and red cell potassium were complementary. This is worth noting. Evans (1954) showed that sheep with high blood potassium levels had low blood sodium, and those with low blood potassium had high blood sodium. The two classes of sodium however, overlapped. Other writers (Kidwell *et al.*, 1959) have emphasised that the simple expectation was not realised that the concentrations of these two ions would compensate each other both between and within the LK and HK classes.

In the present analysis, the individual estimates were adjusted for the effect of factors other than the *Ka* genotypes and the expected intimate relationship is exposed in the table. This finding is now in line with proposals of membrane chemists (Tosteson and Hoffman, 1960) in which the primary effect of this genotype is to set the steady-state concentrations of both ions rather than one only. Nonetheless, this relationship was not quite

perfect. It can be seen that the cationic ratio across the cell membrane, that is, variate 11, had a trend across the genotypes and was significantly higher for high K (HK) than for low K (LK) sheep. This appears to be due to the contribution of the intracellular cations. This effect would be false if the magnitude of the interference of potassium on sodium were greater than expected. The data are, however, in accord with the meticulous estimations on four LK and four HK sheep by Duthie (1961) and may therefore indicate the presence of another osmotically active factor in LK red cells.

(b) *The effect of haemoglobin genotypes* (*Hb*) (table 5)

Sheep of *Hb* type *A* have higher average unadjusted values of PCV than type *B* with type *AB* sheep in between (Mounib and Evans, 1959; Evans and Whitlock, 1964; Fechter and Myburgh, 1966). The data of this paper confirm the effect when other factors are allowed for. Mounib and Evans

TABLE 5

Blood values for haemoglobin genotypes, adjusted for other effects

		Mean values			Standard error		
	Variate	*Hb A*	*Hb AB*	*Hb B*	"AV" S.E.*	"MAX" S.E.*	"MIN" S.E.*
1.	K_c	38·01	37·43	37·03	0·394	0·436	0·346
2.	Na_c	55·97	55·46	52·43	1·361	1·508	1·197
3.	K_p	5·250	5·304	5·271	0·0646	0·0715	0·0568
4.	Na_p	136·36	136·33	135·90	0·458	0·507	0·403
5.	Na_c/K_c	2·672	2·747	2·617	0·0985	0·1092	0·0866
6.	Na_p/K_p	27·03	26·56	26·61	0·448	0·497	0·394
7.	K_c/K_p	7·332	7·096	7·030	0·1482	0·1642	0·1302
8.	Na_c/Na_p	0·413	0·410	0·391	0·0108	0·01192	0·0095
9.	Na_c+K_c	93·98	92·89	89·45	1·338	1·483	1·176
10.	Na_p+K_p	141·611	141·631	141·170	0·4625	0·5125	0·4066
11.	$\frac{Na_c+K_c}{Na_p+K_p}$	0·6666	0·6593	0·6378	0·01081	0·01198	0·0095
12.	PCV %	36·79	35·51	34·04	0·250	0·277	0·219
13.	Hb %	13·78	13·76	13·64	0·052	0·057	0·045
14.	MCHC	37·72	38·92	40·28	0·205	0·227	0·180

Variates 1, 2, 3, 4, 9, 10 in mEq./l.; 13 in grams haemoglobin per 100 ml. whole blood.

* See text.

K_c = Red cell potassium. Na_c = Red cell sodium. K_p = Plasma potassium. Na_P = Plasma sodium. PCV = Haematocrit. Hb = Haemoblobin. MCHC = Mean corpuscular haemoglobin concentration.

(1959) also found that *A* cells have a significantly lower dry matter content than *B* cells. In explanation of these facts it will be seen that the differences in amount of haemoglobin per unit volume of blood were slight, but the MCHC differences were marked. Haemoglobin was thus less concentrated in type *A* cells than type *B* with the *AB* intermediate. The suggested explanation would assume that cells of all haemoglobin types had the same average number of haemoglobin molecules, and because of their electrophoretic distinction the *A* molecules would contribute more negative charges than the *B*. For the maintenance of electroneutrality two possible sequelae come to mind; there could be a reduction in the chloride anions, for which we have no data in this work, or an increase in cations. The latter would result in an increase in the number of osmotically active particles in the cell. More

water would thus be needed by *A* cells than by *B* cells to maintain osmotic balance with the plasma. The result would be a relatively greater diluting of the contents of the *A* than the *B* cells. Because the haemoglobin molecule cannot normally pass through the cell membrane the MCHC would be reduced. The data support the supposition that the differences in MCHC were due to differences in the red cell volume (PCV, variate 12).

It would follow from this hypothesis that the steady-state proportion of cell cations to haemoglobin would be higher in *A* cells than *B* with the *AB* intermediate. This expectation is confirmed by the data; the ratio of cell sodium and potassium (variate 9) to haemoglobin concentration was 2·49 for *A* cells, 2·39 for *AB* cells and 2·22 for *B* cells.

These speculations are open to experiment, and it is the intention to find the effects of *Ka* and *Hb* genotypes on cell number, shape, volume and osmotic fragility, some of which would seem to be inevitable.

(c) *The effect of breeding* (tables 6 and 9)

Differences in the red cell potassium concentrations of sheep of different breeds (Meyer, 1963) and of strains within a breed (Turner and Koch, 1961) have been described. Such a difference was not present in the particular breeds of this study. It should be added that our estimates may not be representative of them because of sampling error in the foundation animals of our flock.

Among the pure breeds, differences below the 0·01 probability level lay in the relatively high red cell sodium values for Blackface, and in the high PCV and low MCHC for Welsh. With a somewhat greater risk of chance intervention but of equal interest, there was a similar pattern of breed values for MCHC and for the cationic ratio between cells and plasma (variate 11). It is inferred from these observations that the red cells of the Blackface had more concentrated contents than the Welsh, with Cheviot values lying between, suggesting that there are fundamental differences in red cell economy among these breeds.

When the values for the crossbreds are compared with the mid-parental values (that is to say, the average of values for the two breeds contributing to the cross) it may be simply said that in no comparison was the difference statistically significant (table 9).

(d) *The effect of age* (table 7)

A regular feature is the many significant differences between the first and second age classes. Broadly, it was not until their second year of age that the animals settled into the mature range of estimates. Pregnancy was presumably the reason for this, as the females were not mated until their second year (table 2). The data fluctuated; there were significant differences between years but no trends to arouse interest. Field *et al.* (1969) also noted an irregular effect of age on plasma potassium.

(e) *The effect of sex and management* (table 8)

Although small differences in the variates are apparent, none had statistical significance.

Our results agree with those of Turner and Koch (1961) who found no significant difference between the mean red cell potassium estimate for females and that for males grouped with castrated males. Evans (1961)

TABLE 6

Blood values for breeds, adjusted for other effects

	Variate	Mean values of pure breds			Mean values of crossbreds			Standard errors		
		Blackface (B)	Cheviot (C)	Welsh (W)	B×C	B×W	C×W	"AV" S.E.*	"MAX" S.E.*	"MIN" S.E.*
1.	K_c	38·46	37·71	37·45	37·80	38·01	35·58	0·941	1·130	0·680
2.	Na_c	62·08	55·11	53·35	52·93	50·59	56·86	3·251	3·906	2·349
3.	K_p	5·401	5·187	5·419	5·286	5·197	5·276	0·1542	0·1853	0·1114
4.	Na_p	135·08	135·39	134·94	134·24	137·82	138·07	1·094	1·315	0·791
5.	Na_c/K_c	2·86	2·66	2·67	2·57	2·37	3·11	0·235	0·283	0·170
6.	Na_p/K_p	25·49	26·93	25·01	25·85	27·11	28·56	1·071	1·287	0·774
7.	K_c/K_p	7·09	7·50	7·12	7·28	7·19	6·75	0·354	0·425	0·256
8.	Na_c/Na_p	0·464	0·408	0·401	0·397	0·371	0·415	0·0257	0·0309	0·0186
9.	Na_c+K_c	100·5	92·8	90·8	90·7	88·6	92·4	3·20	3·84	2·31
10.	Na_p+K_p	140·5	140·6	140·4	139·5	143·0	143·3	1·10	1·33	0·80
11.	$\frac{Na_c+K_c}{Na_p+K_p}$	0·720	0·663	0·652	0·654	0·622	0·648	0·0258	0·0310	0·0187
12.	PCV %	33·81	34·54	36·86	33·33	36·30	36·85	0·596	0·716	0·431
13.	Hb %	13·47	13·69	13·91	13·27	13·91	14·07	0·124	0·148	0·089
14.	MCHC	39·91	39·80	37·81	39·94	38·54	38·54	0·489	0·587	0·353

Variates 1, 2, 3, 4, 9, 10, concentrations in mEq./l.; 13 in grams haemoglobin per 100 ml. whole blood.

* See text.

K_p = Red cell potassium. Na_c = Red cell sodium. K_p = Plasma potassium. Na_p = Plasma sodium. PCV = Haematocrit. Hb = Haemoglobin. MCHC = Mean corpuscular haemoglobin concentration.

TABLE 7

Blood values for ages, adjusted for other effects

	Variate	Age 1	Age 2	Age 3	Age 4	Age 5	" AV " S.E.*	" MAX " S.E.*	" MIN " S.E.*
1.	K_c	34·82	38·08	37·88	40·36	39·13	1·544	2·887	0·496
2.	Na_c	50·79	56·00	57·41	54·08	56·14	5·335	9·978	1·714
3.	K_p	5·408	5·274	5·081	5·346	5·337	0·2531	0·4733	0·0813
4.	Na_p	135·30	136·27	135·73	136·85	138·83	1·795	3·358	0·577
5.	Na_c/K_c	2·574	2·854	2·822	2·356	2·674	0·3862	0·7224	0·1241
6.	Na_p/K_p	25·31	26·98	27·88	27·03	26·71	1·757	3·287	0·564
7.	K_c/K_p	6·446	7·154	7·602	7·604	7·366	0·5808	1·0861	0·1865
8.	Na_c/Na_p	0·374	0·417	0·429	0·402	0·407	0·0422	0·0789	0·0136
9.	Na_c+K_c	85·6	94·1	95·3	94·4	95·3	5·25	9·81	1·68
10.	Na_p+K_p	140·71	141·55	140·81	142·19	144·17	1·813	3·391	0·582
11.	$\frac{Na_c+K_c}{Na_p+K_p}$	0·6062	0·6712	0·6830	0·6703	0·6647	0·04236	0·07922	0·01361
12.	PCV %	35·54	35·81	35·00	34·84	35·08	0·978	1·830	0·314
13.	Hb %	13·10	14·07	13·93	13·87	14·07	0·203	0·379	0·065
14.	MCHC	37·11	39·51	40·02	40·10	40·20	0·802	1·500	0·258

Variates 1, 2, 3, 4, 9, 10, concentrations in mEq./l.; 13 in grams haemoglobin per 100 ml. whole blood.

* See text.

K_p = Red cell potassium. Na_c = Red cell sodium. K_p = Plasma potassium. Na_p = Plasma sodium. PCV = Haematocrit. Hb = Haemoglobin. MCHC = Mean corpuscular haemoglobin concentration.

found males to have a higher whole blood value than females; he ascribed this difference to the red blood cells. This inference was based on data from females and any haematocrit difference due to sex or management would be

TABLE 8

Blood values for sex and management, adjusted for other effects

	Variate	Mean values: Female	Mean values: Male	"AV" S.E.*
1.	K_c	37·49	37·18	1·229
2.	Na_c	54·97	52·92	4·249
3.	K_p	5·212	5·612	0·2015
4.	Na_p	135·99	137·22	1·430
5.	Na_c/K_c	2·669	2·784	0·3076
6.	Na_p/K_p	27·00	25·20	1·399
7.	K_c/K_p	7·286	6·382	0·4625
8.	Na_c/Na_p	0·408	0·389	0·0336
9.	Na_c+K_c	92·47	90·10	4·177
10.	Na_p+K_p	141·20	142·84	1·444
11.	$\frac{Na_c+K_c}{Na_p+K_p}$	0·6591	0·6309	0·03373
12.	PCV%	35·395	35·082	0·7791
13.	Hb%	13·681	13·964	0·1615
14.	MCHC	38·89	39·94	0·639

Variates 1, 2, 3, 4, 9, 10 in mEq./l.; 13 in grams haemoglobin per 100 ml. whole blood.

* See text.

K_c = Red cell potassium. K_p = Plasma potassium. Na_c = Red cell sodium. Na_p = Plasma sodium. PCV = Haematocrit. Hb = Haemoglobin. MCHC = Mean corpuscular haemoglobin concentration.

TABLE 9

Deviations of crossbred blood values from the mean values of the parental breeds

	Variate	Deviation: B×C	Deviation: B×W	Deviation: C×W	Standard errors of the deviation: B×C	Standard errors of the deviation: B×W	Standard errors of the deviation: C×W
1.	K_c	−0·290	0·053	−2·000	1·0162	1·2577	1·1879
2.	Na_c	−5·66	−7·13	2·63	3·513	4·347	4·106
3.	K_p	−0·008	−0·213	−0·027	0·1666	0·2062	0·1948
4.	Na_p	−1·00	2·81	2·91	1·182	1·463	1·382
5.	Na_c/K_c	−0·188	0·389	0·448	0·2543	0·3147	0·2972
6.	Na_p/K_p	−0·35	1·86	2·59	1·157	1·432	1·352
7.	K_c/K_p	−0·018	0·088	0·562	0·3824	0·4732	0·4469
8.	Na_c/Na_p	−0·0387	−0·0615	0·0103	0·02778	0·03438	0·03247
9.	Na_c+K_c	−5·95	−7·07	0·64	3·454	4·274	4·037
10.	Na_p+K_p	−1·00	2·60	2·88	1·194	1·477	1·395
11.	$\frac{Na_c+K_c}{Na_p+K_p}$	−0·0377	−0·0639	−0·0095	0·02789	0·03451	0·03260
12.	PCV %	−0·85	0·97	1·15	0·644	0·797	0·753
13.	Hb %	−0·309	0·217	0·272	0·1335	0·1652	0·1560
14.	MCHC	0·085	−0·317	−0·262	0·5280	0·6535	0·6172

Variates 1, 2, 3, 4, 9, 10 in mEq./l.; 13 in grams haemoglobin per 100 ml. whole blood.

* See text.

K_c = Red cell potassium. Na_c = Red cell sodium. K_p = Plasma potassium. Na_p = Plasma sodium. PCV = Haematocrit. Hb = Haemoglobin. MCHC = Mean corpuscular haemoglobin concentration.

unseen. The data of our paper give no support for such a distinction and Schalm (1965) had found no data showing a sex difference in PCV of sheep. The present opinion would be that variation in red cell potassium concentrations due to sex/management differences is not exhibited by all flocks of sheep.

Sex/management was also confounded with stage of pregnancy (table 2) but less than half of the females had been pregnant for 60 days and more: so any differences associated with a later stage of pregnancy may well have been obscured. They may, however, have shown up as batch differences.

(f) *The effect of batch*

When the adjusted estimates of the variables were plotted in batch sequence some of the curves, especially those of the sodium estimates, showed enough variation to prevent a simple description, while others clearly approximated a straight line. The regressions of the weekly estimates of each variable on time were calculated. For economy of presentation, these regression coefficients (to the first degree only) which describe the data well (computed t greater than 2·4) are given in table 10. In that table it

TABLE 10

The trends of certain blood parameters estimated on 21 occasions from December to June. From data adjusted for other effects

		Regression coefficient	Standard error	Weekly rate of change
PCV	%	−0·305	0·05916	−0·861%
MCHC	%	+0·311	0·03487	+0·796%
K_c	mEq./l.	+0·498	0·09860	+1·334%
$\frac{Na_c+K_c}{Na_p+K_p}$		+0·00494	0·002040	+0·756%

will be seen that over the period of December to June the packed cell volume declined, but the MCHC and red cell K estimates, and the cationic ratio of cell Na+K to plasma Na+K rose, at rates of about 1 per cent. per week.

In table 11 the same phenomenon is expressed as a negative correlation

TABLE 11

The correlation of packed cell volumes with certain blood parameters in samples drawn on 21 occasions from December to June. From data adjusted for other effects

	K_c	$\frac{Na_c+K_c}{Na_p+K_p}$	MCHC
PCV	−0·752	−0·612	−0·736
MCHC	0·629	0·343	

between the weekly adjusted values for PCV and the concentrations of haemoglobin, Na and K, per unit volume of cells. These data are in accord with the suggestion that the red cells shrank in volume, and their contents became more concentrated as the season advanced; perhaps as the result of an increase in osmotic pressure of the plasma. The contribution of the plasma Na and K to this increase appears to have been negligible. We have

evidence that packed cell volumes declined during pregnancy but an exploration of these relationships would lead us away from the subject of this paper.

In conclusion we hope to have shown that these simple Mendelian factors imposed complex sequelae on the red cells. These were, however, largely obscured by familiar variations in breed, age and sample batch.

Acknowledgments.—It is a pleasure to thank Mr Harris and his men for handling the sheep, Mrs Rosemary Marwick for help with punched cards, Mrs Maureen Hamilton and Mrs Meryll Osler for laboratory work, Dr E. J. Butler for the original instruction in making the electrolyte estimations, Dr I. A. Nimmo for valuable criticism of this paper and Mr H. D. Patterson for his help with the analysis.

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