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EFFECT OF FREQUENCY OF HANDLING SHEEP ON BLOOD CONSTITUENTS, WITH SPECIAL REFERENCE TO POTASSIUM AND SODIUM AND THE REPEATABILITY OF THE ESTIMATES

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SUMMARY

Of 18 blood constituents, only white cell, lymphocyte and eosinopil counts, plasma sodium, erythrocyte potassium and blood inorganic phosphorus showed significant changes due to frequent *v.* infrequent handling of Merino sheep. Of these, the blood inorganic phosphorus values showed the greatest change.

The repeatabilities of the estimates from this population of 56 sheep sampled on 2 occasions 8 weeks apart were calculated. Highly repeatable estimates were obtained for haemoglobin, packed cell volume, red cell count, white cell count, neutrophils, lymphocytes, eosinophils, erythrocyte and blood potassium, plasma globulin and total protein and blood inorganic phosphorus.

The possibility that the non-repeatable estimate of erythrocyte sodium may make it difficult to assign a sheep to a particular Kerr classification is demonstrated.

I. INTRODUCTION

Variations in haematocrit values for sheep (Thomson, Thomson and Cuthbertson 1946; Turner and Hodgetts 1959) and in a number of blood constituents for cattle (Gartner, Ryley and Beattie 1965; Gartner, Callow, Granzien and Pepper 1969) have been reported in relation to excitation and handling of the animals. Gartner, Granzien and Murray (1968) recorded data on a number of constituents in the blood of Merino sheep sampled every 2 months for 2 years. On sampling days the animals were mustered, confined in a shed, bled and thereby subjected to some degree of stress. In the period between sampling, the sheep grazed undisturbed over extensive areas and the effect of handling sheep at more frequent intervals on the values obtained could not be investigated. The present experiment was done to examine the effect of frequent *v.* infrequent handling of sheep on certain blood constituents and to determine the repeatability of the estimates.

II. MATERIALS AND METHODS

Animals and treatments.—Fifty-six mature ewes of Medium Peppin origin which had not previously been bled were selected from the flock at the Animal Research Institute, Yeerongpilly, Brisbane for this experiment. Their mean (± SD) initial body-weight was 33·5 ± 2·89 kg. They were drenched to control internal parasites 1 week before the commencement of the experiment and again 6 weeks later. For the initial bleeding, the sheep were brought in from a paddock, confined in a shed, weighed then bled in random order.

They were allocated at random into two groups of 28 animals on a body-weight basis. Each group was assigned to a paddock and they were rotated between the two paddocks with a grazing period of 10 days in each. The treatments were a frequently handled group which was brought to a shed and handled as if being bled 3 days a week for 8 weeks and an infrequently handled group which, apart from drenching and rotation between paddocks, was undisturbed. Holding the sheep involved straddling the animal for approximately 1 min and holding the head firmly to one side while exerting some pressure on the external jugular vein with the thumb. Both groups were bled again 8 weeks from the initial sampling.

Methods.—The analytical methods employed were as follows: haemoglobin (Hb), Donaldson *et al.* (1951); PCV by the microhaematocrit method using a relative centrifugal force of approximately 7,200 *g* for 8 min; red cell count (RCC) and total white cell count (WCC) using an automatic cell counter*; absolute neutrophil, lymphocyte, monocyte and eosinophil counts, Granzien (1968); mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) by calculation; plasma sodium [Na^+p], blood sodium [Na^+b], plasma potassium [K^+p] and blood potassium [K^+b] by flame photometry†; erythrocyte sodium [Na^+e] and erythrocyte potassium [K^+e] were calculated by difference using the respective values for blood, plasma and PCV; plasma total protein, albumin and globulin, Gornall, Bardawill and David (1949); and blood inorganic phosphorus (BIP), Moir (1954).

Plasma for [Na^+p] and [K^+p] estimation was separated from whole blood by centrifugation within approximately 30 min of bleeding.

In the statistical analysis of results, covariance analyses were used to correct for initial values. An overall analysis was done to estimate the repeatability of measurements (Fisher 1946).

III. RESULTS

The results are summarized in Table 1.

One sheep from the infrequently handled group had [K^+b] and [K^+e] concentrations at the 2 samplings, of 20·7, 20·0 and 61·6, 61·1 respectively, with corresponding values for [Na^+b] of 110·0, 116·0 and for [Na^+e] of 32·2, 41·4. As these values were markedly different from those found in the other 55 sheep, results for Na and K from this sheep were excluded from the statistical analyses. Values for other blood constituents from this sheep were not significantly different from the population and were included.

*Celloscope 101, AB Lars Ljunberg & Co.
†IL flame photometer Model 143, Instrumentation Laboratory Inc.

TABLE 1

MEANS AND SIGNIFICANT DIFFERENCES FOR BLOOD CONSTITUENTS RELATIVE TO THE HANDLING OF SHEEP AND REPEATABILITY OF ESTIMATES

Determination	Frequently Handled Sheep	Infrequently Handled Sheep	Average S.E. of Group Means	Repeatability
Hb (g/100 ml)	9·85	9·78	0·12	0·644**
PCV (%)	34·20	33·66	0·48	0·542**
RCC (10^6/mm^3)	7·33	7·33	0·10	0·519**
MCV (μ^3)	46·56	46·22	0·61	0·230*
MCHC (%)	29·02	28·96	0·34	0·254*
WCC (10^3/mm^3)	8·85	10·05*	0·36	0·586**
Neutrophils (10^3/mm^3)	2·60	2·98	0·20	0·519**
Lymphocytes (10^3/mm^3)	5·38	6·27**	0·22	0·834**
Monocytes (per mm^3)	52·9	67·6	13·4	0·064
Eosinophils (per mm^3)	787·6	607·9*	56·9	0·506**
[Na + *p*] (m-equiv./l.)	142·5	143·9**	0·30	0·247*
[Na + *e*] (m-equiv./l.)	102·5	99·4	1·48	0·213
[Na + *b*] (m-equiv./l.)	128·6	128·8	0·48	0·264*
[K + *p*] (m-equiv./l.)	4·54	4·43	0·04	0·175
[K + *e*] (m-equiv./l.)	10·56	11·65**	0·23	0·912**
[K + *b*] (m-equiv./l.)	6·63	6·83	0·08	0·898**
Plasma albumin (g/100 ml)	3·84	3·74	0·07	0·205
Plasma globulin (g/100 ml)	3·66	3·57	0·11	0·433**
Total plasma protein (g/100 ml)	7·50	7·31	0·09	0·414**
BIP (mg/100 ml)	3·01	3·62**	0·10	0·528**

* Significant at $P < 0{\cdot}05$. ** Significant at $P < 0{\cdot}01$.

Despite randomization of the sheep to treatments, there were significant differences between the groups in mean initial values for eosinophil ($P<0{\cdot}01$) and BIP ($P<0{\cdot}05$) and differences for lymphocytes approached significance ($0{\cdot}10>P>0{\cdot}05$). Thus values at the final bleeding (Table 1) were corrected for initial values by covariance.

Handling did not significantly affect the following constituents: Hb, PCV, RCC, MCV, MCHC, neutrophils, monocytes, [Na+*e*], [Na+*b*], [K+*p*], [K+*b*], plasma albumin, globulin and total protein. Results for both groups of sheep were combined in measuring the repeatability of estimates. With the exception of monocytes, [Na+*e*], [K+*p*], and plasma albumin, there were significant repeatabilities for all the blood constituents analysed. For [K+*e*] over 90% of the variation was due to the sheep and only the small remainder of the variation due to external circumstances.

IV. DISCUSSION

Thomson, Thomson and Cuthbertson (1946) showed that the elevation of PCV through exercise could be minimized by quiet handling of the sheep for some time before sampling. Gartner, Callow, Granzien and Pepper (1969) found that daily simulation of the bleeding procedure with cattle for a week conditioned them to the extent that stable values were obtained for blood constituents which otherwise were affected by excitation. Under the conditions of the present experiment, simulating bleeding procedures in sheep 3 days a week for 2 months affected WCC, lymphocyte, eosinophil, [Na+*p*], [K+*e*], and BIP values only. Although these differences were statistically significant, in our opinion they were of dubious biological significance.

As far as WCC and differential WCC are concerned, the triad of neutrophilia, lymphopenia and eosinopenia is usually associated with stimulation or stress (Schalm 1965). This was not evident in the infrequently handled sheep. Although the eosinophil counts were significantly lower, the higher WCC were due mainly to an increase in lymphocytes, whereas there was no difference in neutrophils. It does not appear therefore that the sampling of infrequently handled sheep produces a stress effect on the leucocyte values.

The difference of 1·4 m-equiv./l. in [Na^+p] due to handling is proportionately not great. Gartner, Ryley and Beattie (1965) found no consistent pattern in changes recorded for [Na^+p] in cattle due to degrees of excitation. The magnitude of the change in [K^+e] of approximately 1 m-equiv./l. is considered relatively unimportant in any work defining the values for this particular blood constituent due to the very large differences which have been shown to exist between sheep in [K^+e] (Evans 1957, 1961; Turner and Koch 1961).

The difference in BIP of 0·6 mg/100 ml due to treatment is similar to the absolute difference found in blood samples of cattle taken either by venipuncture in the normal manner or unobserved through a cannula when the animals were at rest (Gartner, Ryley and Beattie 1965). Of all the determinations done on our sheep, BIP showed proportionately the greatest change due to the effects of handling. However, with the exception of this determination, it is concluded that the magnitude of a number of blood constituents, particularly red cell parameters, would not be markedly affected by the fact that grazing sheep were handled only once over 2 months for bleeding.

When the values for blood constituents are compared with data from younger sheep of the same breed extensively grazing on Mitchell grass downs in north-western Queensland (Gartner, Granzien and Murray 1968), certain differences are apparent which cannot be accounted for as being due to an effect of age. Values for Hb, PCV, RCC and [Na^+p] were lower and values for WCC, lymphocytes and [Na^+e] were higher in the sheep sampled in Brisbane.

Four distinct groups of sheep exist depending on the concentration of [Na^+e] and [K^+e] (Evans 1957) and have been designated $Ke\infty$, $Ke\beta$, $Ke\gamma$ and $Ke\Delta$ Although Evans (1957) showed that neither the intravenous injection of adrenaline, severe exercise and excitement, nor anaethetization had any significant effect on the concentration of [K^+e], and this is supported by the high repeatability figure for this estimate in the present work ($r = 0{\cdot}912$, $P<0{\cdot}01$), there were no data showing the same is true for [Na^+e]. Evans and Phillipson (1957) suggested that [Na^+e] is a more labile value compared with [K^+e]. Koch and Turner (1961) in studies with lambs found the adult sheep values for [Na^+e] and [K^+e] were reached on the average by 60 days of age and beyond 60 days values for [Na^+e] appear to fluctuate more than those for [K^+e], but as only a few figures were available, no definite conclusions were drawn.

We found [Na^+e] to be one of the few estimates that was not repeatable and this could under certain circumstances make it difficult to fit sheep into one of the groups mentioned by Evans (1957). To illustrate this, the [K^+e] of 61 m-equiv./l. found in the one atypical sheep suggests it is more likely $Ke\beta$ or $Ke\Delta$ which are relatively common in Middle East breeds (Kerr 1937) and have been shown to exist in fine-wool Merino sheep (Evans 1957). This sheep had a [Na^+e] value of 32 m-equiv./l. on one occasion, suggesting a $Ke\Delta$ grouping, and on the second occasion a [Na^+e] value of 41 could indicate a $Ke\beta$ grouping. Although Merino sheep can be accurately differentiated as high or low K on

the basis of [K^+e], further subdivision on the basis of [K^+e] and [Na^+e] could lead to error, particularly on the basis of a single sample. Recent findings (Evans, Agar and Roberts 1970) suggest that the atypical sheep was probably not a true Δ type animal because it did not have an unusually high MCHC.

The mean [K^+e] of approximately 11 m-equiv./l. found in the 55 sheep was similar to that found in other Merino sheep by Denton *et al.* (1951), Harris, McDonald and Williams (1952) and Evans (1957, 1961). The incidence of a high [K^+e] in 1 Merino sheep out of 56 compares with 8 out of 1655 (Evans 1961), 8 out of 366 (Turner and Koch 1961), nil out of 28 (Gartner, Granzien and Murray 1968) and nil out of 60 (Gartner, unpublished data).

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