THE EFFECT OF FEEDING AND SALIVATION ON ACID–BASE STATUS IN CAROTID AND JUGULAR BLOOD IN SHEEP

By A. W. BILLITZER* and I. G. JARRETT*

Summary

The Astrup procedure has been used to assess the acid–base status of sheep blood by determination of base excess in carotid arterial and jugular venous samples.

When assessing acid–base status in sheep, it is important that the results be interpreted in relation to time of feeding and site of sampling.

When sheep are fed once hourly a "steady state" is achieved and there is no diurnal variation in either the carotid or jugular blood, although the base excess of jugular venous blood is slightly lower than that of carotid arterial blood.

Sheep fed once daily and consuming their ration within 2 hr, however, show a marked fall in base excess of jugular vein blood during feeding. This phenomenon appears to be associated with salivary flow.

It is suggested that these changes are associated with the movement of bicarbonate, and a possible cycling of bicarbonate is discussed in relation to acid–base status in ruminants.

I. INTRODUCTION

Determinations of the acid–base status in humans can be made with very small samples of blood using a method developed by Astrup (1957) and nomograms devised by Siggaard Andersen and Engel (1959). Compared with the large volume of data accumulated on human blood, only a few reports have appeared on the acid–base status of sheep (Katz and Bergman 1966; Stacy 1969) and no extensive studies appear to have been reported on the use of the Astrup procedure with this species.

The current study was undertaken to establish the suitability of the Astrup procedure and the use of the Siggaard Andersen nomograms for sheep's blood obtained from a jugular vein and a carotid artery. Some aspects of acid–base status in relation to feeding in ruminants were studied during periods when large volumes of saliva are secreted.

II. EXPERIMENTAL PROCEDURE

Adult Merino ewes aged 3 yr were used, either while confined in metabolism cages to which they had become accustomed or while housed in individual pens. Water was available at all times and a ration of 750 g wheaten hay chaff and 250 g lucerne chaff was fed. Feed was offered either once a day or in equal amounts at intervals of 1 hr by automatic feeder. The animals fed once a day were trained to eat their ration within 2 hr and those fed by automatic feeder consumed their hourly portion within the allotted time spans.

Midway between each hourly feed, blood samples were taken from the left jugular vein and carotid artery over a period from 8.00 a.m. to 10.00 p.m. On any one day two sheep were sampled and this procedure continued for a total period of 10 days thus giving, for statistical analysis, a total of five sets of samples from each of four animals.

* Division of Nutritional Biochemistry, CSIRO, Adelaide, S.A. 5000.

Venous blood samples were taken from the jugular vein either by venipuncture with a 19-gauge needle or from an in-dwelling cannula (PVC, 0·8 mm int. diam.) when repeated samples over several days were being taken. A cannula sufficiently long to be attached to the back of the animal enabled samples to be taken with a minimum of disturbance. The cannula was flushed with isotonic NaCl solution and filled with heparin (5000 units/ml) to reduce any tendency for clot formation in the lumen when not in use.

In four animals a carotid artery was exteriorized in a loop of skin, and in some of the studies samples were obtained from this vessel simultaneously with samples from a vein.

Blood (5 ml) was drawn into a 10-ml glass Luer-Lok syringe in which the dead space was filled with heparin (1000 units/ml) and a stainless steel ball (3·2 mm diam.) which acted as a stirrer. After the syringe was sealed, by capping with a needle hub (needle removed and replaced by a spot of solder), it was up-ended several times for mixing and immersed in crushed ice. The sample was thereby cooled in less than 1 min from withdrawal. It was found advisable to mix again before taking samples for processing, thus diminishing any errors associated with temperature gradients throughout the sample.

The micro-electrode of the Astrup Radiometer equipment* was filled by dipping the plastic capillary into the body of the syringe through the needle socket. The equilibration tubes of the tonometer were cleanly filled by attaching a needle to the syringe and inserting it to the bottom of the tubes. The blood was then equilibrated with 3·5 and 6·5% CO_2 in oxygen and the procedure followed according to Astrup (1957). With these precautions the procedure gave highly reproducible results from any one sample.

The buffering efficiency of sheep's blood was tested in relation to the validity of the nomogram of Siggaard Andersen and Engel (1959) by adding acetic acid to give 5 and 10 m-equiv/l of blood (25 and 50 μl 1N acetic acid to 5 ml blood) and the shift in the buffer line on the curve nomogram (Fig. 1) determined.

To establish diurnal variations in relation to feeding patterns, blood samples were taken at various times throughout the day. The acid–base status was assessed by using the concept of base excess (BE) as defined by Siggaard Andersen (1966), where BE is used as a measure of the excess or deficit of base. The BE value indicates the total excess of base but does not indicate whether the condition is the result of addition of base or removal of non-volatile acid. A negative value of BE signifies a base deficit or an excess of non-volatile acid. During conditions of homeostasis a BE equal to zero is accepted as the normal value; for sheep's blood this is equivalent to 26 m-equiv. HCO_3^- per litre (Katz and Bergman 1966; Stacy 1969).

To test the effect of salivation on the BE of jugular vein blood, three sheep were injected subcutaneously with 6 mg atropine at hourly intervals for 4 hr and a week later with 34 mg pilocarpine at hourly intervals for 3 hr. The effect of this amount of atropine or of pilocarpine was assessed in two other sheep in which a parotid fistula had been established. Atropine at the dose rate used reduced salivary flow from a pre-injection level of 20 drops per minute to 1–2 drops per minute and pilocarpine increased the basal rate of 20 drops per minute to 40 drops per minute. An average of six counts, each of 1 min duration, was taken at intervals throughout the period of observation as a measure of salivary flow.

III. Results

(a) Use of the Siggaard Andersen Nomograms for Sheep Blood

Two nomograms, a curve nomogram and an alignment nomogram, have been published by Siggaard Andersen (1966). The curve nomogram is presented in Figure 1 in a simplified form, with the data obtained from a blood sample taken from the jugular vein of a normal sheep plotted as a buffer line (A). When the blood was made 5 m-equiv/l with respect to acetic acid the intercept of the buffer line on the BE curve shifted by the equivalent amount (5 m-equiv.) to position B, and when

* Type E5021, Radiometer A/S-72.Emdrupvej, Copenhagen NV-Denmark.

10 m-equiv/l were added the intercept was shifted to C by the equivalent amount. The shift following the addition of these amounts of acid is in agreement with the theoretical shift of the line on the nomogram as found by Astrup (1961) for human
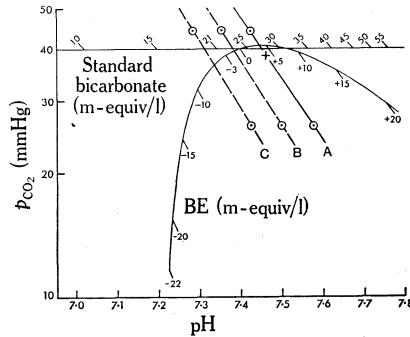

Fig. 1.—Siggaard Andersen curve nomogram with a buffer line for a normal sheep (line A) and the shift to a more negative base excess (BE) on addition of acetic acid to the blood sample (lines B and C).

blood and indicates that the nomogram devised by Siggaard Andersen and Engel (1959) is also applicable to sheep's blood.
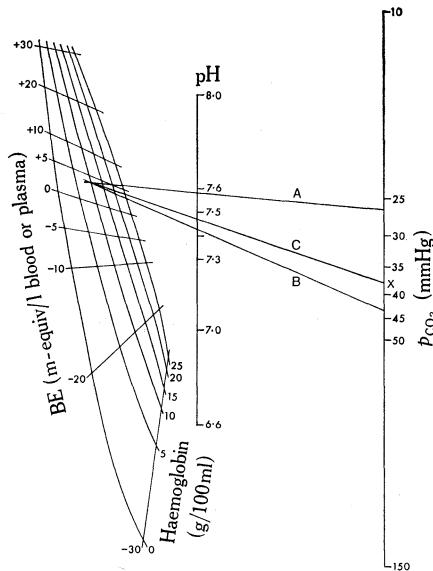

Fig. 2.—Siggaard Andersen alignment nomogram showing the intercept of two equilibration lines on the haemoglobin scale (lines A and B) and check on actual p_{CO_2} using actual pH (line C) (values used are the same as for Figure 1).

Figure 2 shows a simplified version of the alignment nomogram. An advantage in using this for assessment of acid–base status is that the haemoglobin scale can be used as a cross-check on the reliability of the sample used.

As the actual pH was easily affected by extraneous factors of an environmental nature, a more stable fixed reference point was needed to evaluate results in relation to possible sampling errors or changes in blood before measurements were made.

A haemoglobin value of 12 ± 2 g/100 ml blood (Schalm 1965) was chosen as this fixed reference point. The value can be determined spectrophotometrically and the Siggaard Andersen alignment nomogram contains a sufficiently large haemoglobin scale to be read with good accuracy.

When the equilibration values for a blood sample from a normal sheep at the two CO_2 tensions (27 and 44 mmHg) and the corresponding pH values are plotted on the alignment nomogram, the intercept of the two lines A and B so obtained should fall on or close to the haemoglobin lines of 12 ± 2 g/100 ml blood (Fig. 2, lines A and B). Only if the corresponding pH values gave lines which intersected within the range of haemoglobin taken as reference value was the sample used. As the curve nomogram is normally used to derive information on the acid–base status a cross-check from this data can thus be made on the alignment nomogram. Also, when a line is taken from the haemoglobin value (obtained as described above) through the actual pH of the same blood sample (e.g. $7\cdot475$ as shown in Fig. 2, line C) the actual p_{CO_2}
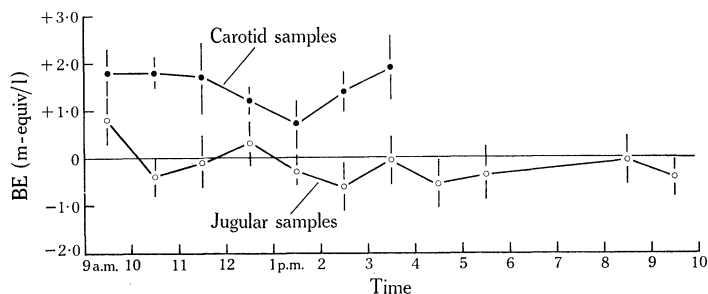

Fig. 3.—Base excess in the carotid blood (●) and in the jugular vein blood (○) for sheep fed once hourly (mean values $\pm$ S.E.M., four sheep).

value obtained from its intercept (X, Fig. 2) should be of the same order as that obtained by the intercept of pH on the buffer line on the curve nomogram (Fig. 1, line A, point X).

The haemoglobin values as determined above from 60 acid–base measurements on three sheep gave a value of 12 ± 2 g/100 ml (mean$\pm$standard error of the mean).

(b) Effect of Feeding on Acid–Base Status in Sheep

(i) Animals Fed every Hour

In order to test for differences between hourly means an analysis was carried out in which variation between sheep and days was first removed from the data. This analysis showed that the variation between hourly means was not significantly greater than the random variation associated with the measurement of base excess. The standard deviation of these measurements was $1\cdot02$ m-equiv/l.

The data is presented in Figure 3 in such a manner that the mean at each time is given with a standard error which includes variation associated with days and with

sheep. No significant difference was observed throughout the day in samples taken from either the jugular vein or the carotid artery, although the arterial sample was consistently higher than the venous sample.

(ii) *Animals Fed Once a Day*

As with the animals fed hourly, a variation between sheep and days was apparent. However, when analysed on the same basis as the sheep fed once daily, and the means at each hour with a standard error plotted (Fig. 4), it can be seen that a change in BE occurred in the first few hours after the feed was consumed. This was particularly noticeable in samples taken from the jugular vein, when the mean value dropped from a BE of +3·2 before feeding to a value of −1·4 by the time the feed had been consumed (2 hr). By midday the BE of the jugular vein blood had risen to
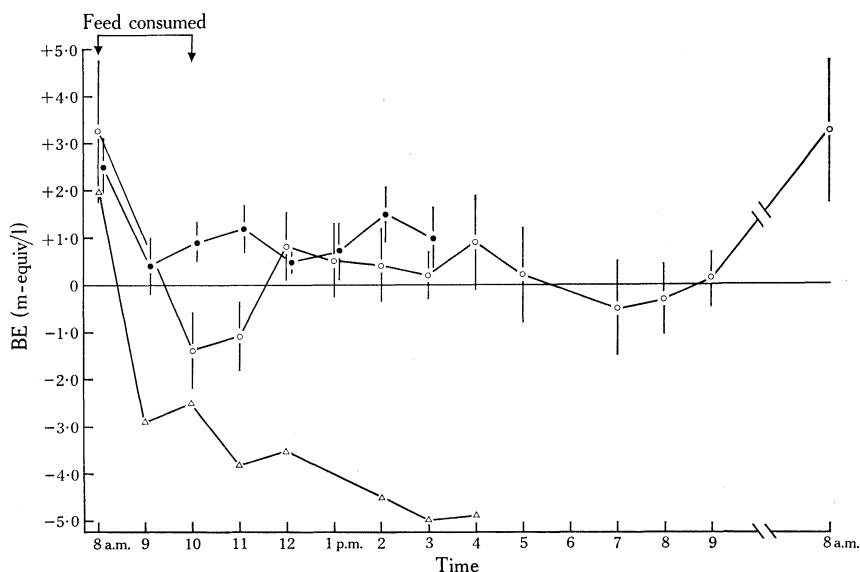

Fig. 4.—Base excess in carotid blood (●, mean value ± S.E.M., four sheep) and jugular vein blood (○, mean value ± S.E.M., six sheep) for sheep fed once daily. △ Values for a jugular vein sample from a placid sheep also fed once a day.

just above zero, at which stage it was not significantly different from that of the carotid artery. At no time did a fall to negative BE values occur in arterial blood.

Occasionally values below the normal lower range were observed in two animals which were particularly placid and spent a large proportion of their time lying down and ruminating immediately after the feed was eaten. The values for one such sheep, not included in the means of the group above, and sampled from a jugular vein cannula while lying down undisturbed over a 5-hr period, are also shown in Figure 4.

(c) *Base Excess of Blood in the Jugular Vein following Injection of Atropine*

It was considered that the drain of bicarbonate into saliva might account for the fall of BE of jugular blood to negative values associated with the rapid consumption of food. In three sheep salivary flow was severely restricted by subcutaneous

injections of 6 mg atropine at the time of feeding and at hourly intervals for 3 hr. The mean values±standard error of the mean for jugular vein blood are shown in Figure 5(a). During treatment with atropine no fall in BE occurred into negative values, despite the animals eating during this period; however, 4 hr after the last injection of atropine a significant fall to negative values took place.

(d) Base Excess of Blood in the Jugular Vein following Injection of Pilocarpine

In three unfed sheep salivary flow was stimulated by three subcutaneous injections of 34 mg pilocarpine at hourly intervals. The mean values±standard error of the mean for jugular vein blood are shown in Figure 5(b), together with the values for another animal which was not fed throughout the period of observation, and did not have pilocarpine. It was apparent that, coincidental with a copious salivary secretion, a marked fall in BE occurred. A return to the pre-injection fasting level (BE+2·5) was observed 2 hr after the last injection.
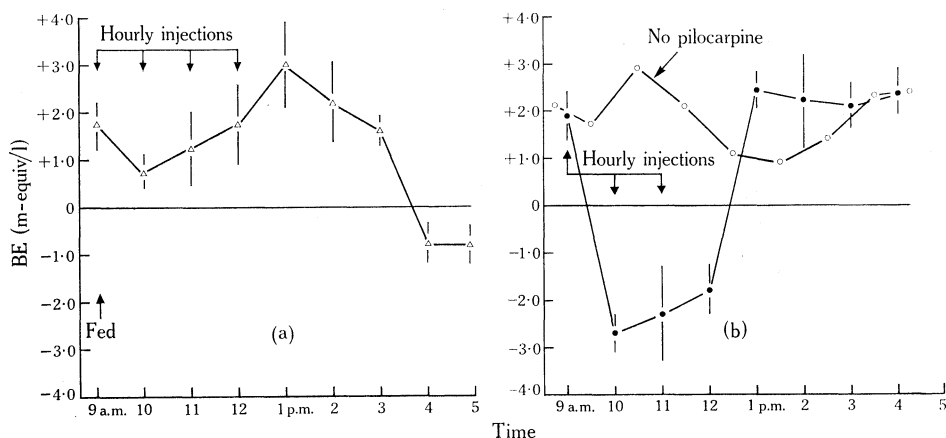

Fig. 5.—(a) Effect of atropine on the base excess in the jugular vein blood of sheep fed once daily (mean values±S.E.M. for three sheep); (b) effect of pilocarpine on the base excess in the jugular vein blood of fasted sheep (●, mean values±S.E.M. for three sheep). Values for a normal fasted sheep (○) are also shown for comparison.

(e) Variations of Base Excess within the Jugular Vein

From the previous data it appeared that salivation might have been the cause of the lower BE in the jugular blood. Therefore the closer the point of sampling in the jugular vein to the venous effluent draining a gland actively secreting saliva, the greater the difference expected in BE between jugular and carotid samples.

To test this, two sheep each had one cannula inserted into the left carotid artery and two cannulae into the left jugular vein. One cannula was passed cranially so that the tip was close to the venous outflow of the parotid gland, the other was passed caudally so that the tip was near the superior vena cava where mixed venous blood from other upper parts of the body could be obtained. Simultaneous samples were taken through each cannula from these animals when fed once a day and when fasted during the effects of pilocarpine. As between-animal variation was marked, each animal was assessed individually and the values for each sampling site for both

conditions for one animal only were plotted [Figs. 6(a), 6(b)]; however, both animals showed the same trend. It is apparent that a sample of blood taken from a jugular vein near the parotid gland has a lower BE than a sample from either lower down the vein or from the carotid artery. Moreover, excessive salivation accentuated this difference.
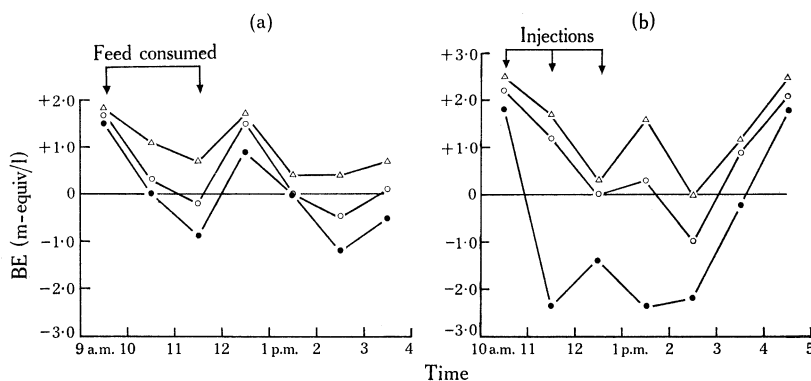

Fig. 6.—(a) Difference in base excess between carotid blood (△), caval blood (○), and jugular blood (●) in a sheep fed once daily; (b) effect of pilocarpine on base excess in carotid blood (△), caval blood (○), and jugular blood (●) of a fasted sheep.
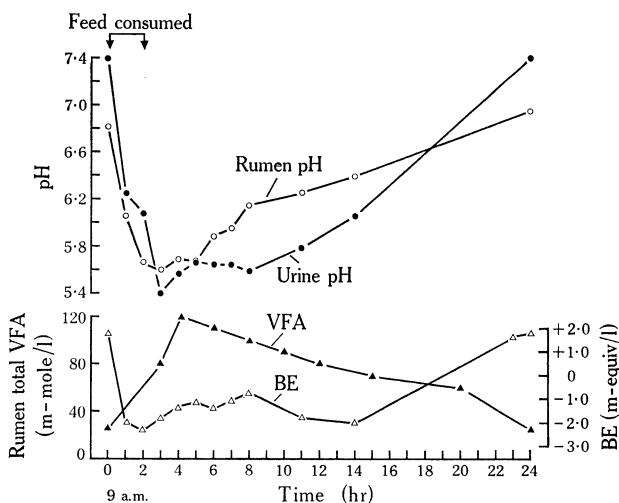

Fig. 7.—Interrelationship between base excess of jugular blood (△), urine pH (●), rumen pH (○), and volatile fatty acids (▲) in a sheep fed once a day.

As a depletion of bicarbonate from the afferent blood to a salivary gland needs replacement, this loss can be made up by bicarbonate reabsorption in the kidney with consequent fall in urine pH. Figure 7 shows the relationship between rumen production of volatile fatty acids, rumen pH, urine pH, and jugular vein BE in an animal fed once daily.

IV. Discussion

Recent work indicates the importance of maintaining a "steady state" in ruminant digestion for the assessment of many parameters which could vary markedly depending on the rate of production and absorption of volatile fatty acids from the rumen (Leng 1970). Minson and Cowper (1966) reported that large variations in the excretion of dry matter and nitrogen in faeces and urine respectively did not occur when sheep were changed from a once-daily to a once-hourly feeding regime.

The lack of diurnal variation in acid–base status of both jugular venous and carotid arterial blood in sheep on an hourly feeding regime is consistent with the relative stability of other parameters previously investigated. Although no significant difference occurred throughout the day in BE of either arterial or jugular venous blood, the consistently higher values of the carotid samples could indicate a steady withdrawal of base between this vessel and the jugular vein. Therefore, in view of the consistency of the BE values in animals feeding throughout the 24 hr, samples taken from either the jugular vein or carotid artery should be adequate to assess the status of the whole animal, with the proviso that in sheep the jugular venous BE is slightly lower than that of the carotid artery.

However, when a sheep is fed once daily and food is consumed rapidly, it is obvious that a sample taken from a jugular vein during or shortly after eating would indicate a much lower BE than would be the case if an arterial sample were taken.

The marked fall in BE of jugular venous blood following pilocarpine administration and the absence of an expected post-prandial fall following atropine injections in a feeding animal indicate the important role of salivary secretion in influencing acid–base status. Moreover, the lower BE in blood taken from a site close to the venous drainage of a parotid gland compared with that of blood mixed with other venous drainage sites provides further evidence that a lower BE may be associated with parotid gland activity.

A progressive increase in the concentration of bicarbonate in sheep saliva with increasing rate of secretion has been reported (Coats and Wright 1957; Obara et al. 1967), and at secretion rates of 100 ml/hr, up to 200 m-equiv/l of bicarbonate could be present in saliva (Coats and Wright 1957). The parotid saliva provides the major fraction of the total bicarbonate in the mixed saliva of sheep and even in spontaneously secreted basal saliva bicarbonate may be about 80 m-equiv/l (Coats and Wright 1957). During the first 30 sec of stimulation the gland is in negative balance with respect to bicarbonate due to initial loss of ion. However, when stimulation continues the tissues of the secreting gland are in bicarbonate balance and the rate of replenishment (i.e. rate of extraction of bicarbonate from the blood) is then roughly equal to outflow of bicarbonate in saliva (Coats, Denton, and Wright 1958). Thus, accepting a basal rate of 80 m-equiv/l of bicarbonate in saliva, when the parotid gland is in bicarbonate balance at least 80 m-equiv. of bicarbonate for each litre of saliva secreted are being extracted from the arterial blood supply, and this is apparently reflected in the difference of BE between jugular and carotid blood.

Stacy (1969) has discussed a number of parameters in relation to the physiological changes that take place after the rapid ingestion of feed over a 2-hr period and has claimed that the observed post-prandial hypercalciuria is related to changes in acid–base balance. Changes in blood pH ($7 \cdot 445$ before feeding and $7 \cdot 376$ 30 min after

feeding) and blood bicarbonate (26 m-equiv/l plasma before feeding and 22·9 m-equiv/l plasma after feeding) in samples taken from a jugular vein were observed. These values agree with the data reported here for BE, where a reduction of BE by about 4 m-equiv/l was observed in the jugular vein within 1–2 hr after feeding.

Values below the normal range observed in two animals (see Fig. 4) which were particularly placid are considered to be related to rumination and salivary flow. Denton (1957) in a discussion on conditioned salivary reflexes of sheep reported marked differences in salivary flow in a "shy Merino wether" fed once daily, depending on activity of other sheep in adjacent cages. The psychic augmentation of secretion could in some animals involve an increase of 60–100 ml of saliva secreted in 30 min.

From the data presented on the fall in BE which is associated with the rapid ingestion of food, and coincidental with the rise in volatile fatty acid production in the rumen and with the reduction in urine pH, it would seem apparent that the "acid-tide" characteristic of sheep fed once daily (Stacy 1969) is associated with the fall in BE of the jugular blood. The interrelationship of feeding patterns with salivary secretion, kidney function, and acid–base status in sheep would suggest a cyclic movement of bicarbonate and should offer interesting fields for further study.

V. Acknowledgments

VI. References

Astrup, P. (1957).—*Scand. J. clin. Lab. Invest.* **8**, 33.
Astrup, P. (1961).—*Clin. Chem.* **7**, 1.
Coats, D. A., Denton, D. A., and Wright, R. D. (1958).—*J. Physiol., Lond.* **144**, 108.
Coats, D. A., and Wright, R. D. (1957).—*J. Physiol., Lond.* **135**, 611.
Denton, D. A. (1957).—*Nature, Lond.* **179**, 341.
Katz, M. L., and Bergman, E. N. (1966).—*Am. J. vet. Res.* **27**, 1253.
Leng, R. A. (1970).—*Adv. vet. Sci.* **14**, in press.
Minson, D. J., and Cowper, J. L. (1966).—*Br. J. Nutr.* **20**, 757.
Obara, Y., Gomi, K., Ootomo, Y., and Tsuda, T. (1967).—*Tohoku J. agric. Res.* **18**, 125.
Schalm, O. W. (1965).—"Veterinary Haematology." (Baillière, Tindall and Cassel: London.)
Siggaard Andersen, O. (1966).—"The Acid–Base Status of the Blood." 3rd Ed. (Munksgaard: Copenhagen.)
Siggaard Andersen, O., and Engel, K. (1959).—*Scand. J. clin. Lab. Invest.* **12**, 177.
Stacy, B. D. (1969).—*Q. Jl exp. Physiol.* **54**, 1.

Corrigendum

Volume 23, Number 3

p. 524, Table 1, heading referring to columns 5 and 6:
For Geraldton 1966, ‡ *read* Geraldton 1967, ‡