Proc. Nutr. Soc. Aust. (1991) 16

MEASUREMENT OF METABOLISM IN WHOLE ANIMALS AND THEIR TISSUES: AN OVERVIEW

E.F. ANNISON

Summary

The availability of new methods for the continuous monitoring of blood flow and blood oxygen content has led to new approaches to the measurement of metabolism in the whole animal, and in defined tissues.

Continuous measurement of oxygen consumption from cardiac output and the difference in blood oxygen content across the lungs can be combined with complementary techniques which include isotope dilution, and the study of defined tissues by arteriovenous (AV) difference techniques. In addition, increased precision of the measurement of nutrient inflows by portal blood analysis has stemmed from the continuous measurement of portal blood flow by the new procedure based on ultrasonics.

I. INTRODUCTION

The measurement of metabolism in animals began with the use of simple calorimeters by Laplace and Lavoisier in the eighteenth century, as highlighted by the late K.L. Blaxter (1962), whose own achievements have contributed so much to our current understanding of energy metabolism. Sir Kenneth Blaxter was a leading figure in the group of European scientists who from the 'fifties developed improved methods for the measurement of energy expenditure in livestock. The data generated by the improved technology contributed to the development of new feeding systems for livestock, including the widely used ME (metabolisable energy) system (Blaxter 1989). The impetus for sustained effort in this area has been the economic importance of the efficient use of feedstuffs.

In recent decades indirect calorimetry has been widely adopted for the measurement of energy expenditure in animals (McClean and Tobin 1987). The need to house animals in respiration chambers, however, has hindered the simultaneous application of complementary techniques, two of the most important of which are isotope dilution and the measurement of metabolism in defined tissues. Recent developments in technology which make it possible to measure continuously the oxygen consumption of animals without recourse to respiration chambers, ventilated hoods or face masks, has overcome this problem (Giles et al. 1989). A key feature of the new procedure is the use of ultrasonics to measure blood flow precisely, and continuously (Transonics Systems Inc., Ithaca, USA). This advance in technology has greatly increased the precision of two classical physiological procedures which depend on knowledge of blood flow. These are the AV difference procedure for the measurement of substrate uptake or release, and the measurement of nutrient inflows into the portal system by portal blood analysis (Bergman 1990).

The use of isotopes in metabolic studies began in the 'thirties, when Schoenheimer and Rittenberg (1935) used deuterated water to study fat metabolism in mice. A later study with ^{15}N labelled glycine demonstrated for the first time the turnover of protein in tissues (Schoenheimer et al. 1939). The use of stable isotopes in metabolism, however, has always been hampered by the complexity and costs of analysis, and the relative insensitivity of stable isotope detection. The availability of metabolites labelled with the radioactive isotopes ^{14}C and ^{3}H in the 'fifties, however,

Department of Animal Science, University of Sydney, Camden, NSW 2570

had a dramatic impact on two areas of animal metabolism. These were the delineation of the metabolic pathways of key nutrients, and the application of isotope dilution to measure inflows into body pools of the major energy yielding nutrients (glucose, acetate, plasma non-esterified fatty acids (NEFA) and 3-hydroxybutyrate), and a range of amino acids (Annison and Leng 1991).

The quantitative study of tissue metabolism probably began with M.A. Chauveau in France in 1887 (cited by Zierler, 1976), who measured arteriovenous (AV) differences of oxygen, carbon dioxide and glucose across the levator muscle of the upper lip of the horse. This was probably the first application of the principle enunciated by Fick in 1870 (Zierler 1976). When applied to the metabolism of defined tissues, the Fick Principle implies that net substrate uptake or release may be measured as the product of AV difference and blood flow. The AV difference method has been widely used, but the precision of the procedure has been greatly enhanced by the availability of a new system for the continuous measurement of blood flow based on ultrasonics (Transonic Systems Inc., Ithaca, USA).

The AV difference procedure may be combined with isotope dilution to provide metabolic data on both defined tissues, and the whole animal (Linzell and Annison 1975). The power of these combined procedures has been still further enhanced by recent developments which make it possible to combine them with the simultaneous measurement of oxygen consumption in both defined tissues, and in the whole animal.

II. WHOLE ANIMAL METABOLISM

(a) Isotope dilution

Isotope dilution procedures provide the only means of obtaining kinetic data on the transfer and turnover of nutrients in the conscious, largely undisturbed animal (Linzell and Annison 1975). Ruminants, largely because of their economic importance and convenient size with respect to blood volume, have been the subject of intensive study since the 'sixties (see Annison et al. 1967), but it is timely to pay tribute to the late Robert Steele, whose early studies on glucose biokinetics in dogs (Steele et al. 1956), and subsequent theoretical treatment of isotopic data (Steele 1964) encouraged many of us to apply the new technology to ruminant metabolism. The following discussion is confined to the use of ^{14}C or ^{3}H labelled substrates in ruminants.

Isotope dilution procedures are based on the single injection or constant infusion of labelled substrates, followed by the withdrawal of blood at intervals for several hours for the assay of the specific radioactivity (SRA) of circulating substrate (Annison and Leng 1991). An important feature of the technique is the use of labelled substrates of high radioactivity but insufficient mass to provoke a hormonal response to the substrate. Adequate mixing of labelled substrate with the body pool of substrate, and maintenance of relative constancy of pool size, are essential. The sites of both administration of labelled substrate and blood sampling may be of importance for some substrates. Problems have been identified with leucine, for example, which appear to be related to the release of leucine by the lungs and portal drained viscera (Pell et al. 1983). In general, administration of isotopic tracer via a jugular vein catheter located near the heart, and sampling of arterial blood are effective for isotope dilution studies with glucose, acetate, NEFA and 3-hydroxybutyrate.

When ^{14}C labelled substrates are used in isotope dilution studies, measurement of the SRA of $^{14}CO_2$ in expired air, or more simply, in arterial blood, provides a rough estimate of the contribution of substrate to total oxidative metabolism. This approach was used in sheep with a range of substrates which included the major VFA, glucose, NEFA and 3-hydroxybutyrate (Annison et al. 1967), but the sum of the values for the contribution of individual substrates to total oxidative metabolism accounted for only 70% (fed sheep) and 58% (starved sheep) of total CO_2 production. The failure to account for total CO_2 production was attributed to the entry of

^{14}C-labelled carbon into pools of low turnover rate. More detailed studies of these relationships will be facilitated by exploiting the new procedures for the continuous measurement of oxygen consumption, which can be easily adapted to provide data on both total CO_2 production, and the SRA of $^{14}CO_2$ during the simultaneous infusion of ^{14}C labelled substrates.

(i) Glucose biokinetics

Measurement of glucose entry rate in sheep by isotope dilution in the 'sixties (Annison and White 1961; Annison et al. 1967) led to a re-evaluation of the quantitative importance of glucose. At that time several features of glucose metabolism, which included the paucity of alimentary glucose, the relative insensitivity of sheep to insulin and the negligible role of glucose as a carbon source for fatty acid synthesis contributed to the view that glucose, unlike acetate, played a minor role in ruminant metabolism (see Annison 1983). Isotope dilution studies, however, revealed that glucose entry rates in ruminants are only marginally lower than those of fasted non-ruminants (Ballard et al. 1969).

Subsequent studies have confirmed that gluconeogenesis is a major metabolic activity in ruminants in both fed and fasted states, and that as in non-ruminants, insulin is the major hormone controlling glucose metabolism (Weekes 1991).

Several studies have shown that glucose entry rate is well correlated with ME intake (see Annison 1983). The contribution of glucose to total oxidative metabolism, based on the ratio of the SRA of circulating glucose to that of blood $^{14}CO_2$ after the prolonged infusion of ^{14}C-glucose is usually about 8%, in contrast to the much higher value usually observed for acetate (see Annison 1983).

(ii) Acetate biokinetics

Elucidation of the role of acetate in ruminant metabolism was made possible by the use of ^{14}C-acetate to delineate metabolic pathways, and to generate data on rates of entry and oxidation of acetate in the whole animal, and in defined tissues (see Annison 1984).

The production of endogenous acetate by most tissues (see Annison 1984) implies that acetate entry rate is not a measure of the inflow into the portal system of acetate produced in the alimentary tract, and acetate entry rate is only weakly correlated with ME intake. Acetate entry is closely related to arterial acetate concentration in sheep, however, irrespective of their nutritional or physiological status (Pethick and Lindsay 1982).

The contribution of acetate to total CO_2 production in fed goats, ewes and cows is about 21, 26 and 33% respectively (see Annison 1984). In the starved goat, this value fell to about 10%.

The efficiency with which acetate is used in ruminants has been discussed since Armstrong and Blaxter (1957) showed that the energy from acetate infused into the rumen of sheep fed dried grass was used less efficiently than the energy from either propionate or butyrate. Black et al. (1987) have used a model based on simulation analysis to predict that the efficiency of acetate utilization is related to NADPH availability. When the proportion of available energy supplied as acetate is high relative to that supplied by NADPH generating nutrients, the model predicts reduced efficiency of utilization of acetate. The new procedures for the continuous measurement of energy expenditure are ideally suited to examine this problem. In current studies in this laboratory, surgically prepared sheep fed high roughage diets are being continuously infused with acetate (one mole/day) via the portal vein to examine possible effects on energy expenditure.

(b) Measurement of energy expenditure by indirect calorimetry

The difficulty of applying complementary techniques to the study of the metabolism of animals housed in respiration chambers was alluded to earlier. Another major limitation is that the

system is unsuitable for the examination of short-term responses in O_2 consumption. The use of face masks or ventilated hoods permits continuous monitoring of O_2 consumption, but there is evidence that face masks induce stress (Liang et al. 1989) and ventilated hoods expose the head of the animal to relatively high airflows.

An alternative approach to indirect calorimetry based on the direct measurement of O_2 consumption has been recently developed (Giles et al. 1989). Oxygen consumption is calculated, by Fick Principle, from cardiac output measured as blood flow through the pulmonary artery, and the difference in blood O_2 content across the lungs. The stimulus for this approach, which is by no means new, was the availability of greatly improved methods for the continuous measurement of both blood flow, and blood O_2 content. The development of this new approach to energy metabolism, and its utility are discussed in a later contribution to this symposium (Gooden and Giles 1991).

The availability of a procedure for the continuous measurement of energy expenditure in normal animals offers many exciting possibilities for future research. The system has already proved suitable for the study of changes in energy expenditure in heat stressed pigs (Giles et al. 1990). The new procedure could be combined with isotope dilution to study the effects of changes in the balance of nutrients on energy expenditure in the whole animal, and in defined tissues. More obvious applications are the evaluation of the effects of drugs or hormones on energy expenditure, and the use of the system to monitor energy expenditure during controlled exercise (Gooden and Giles 1991).

Energy expenditure in animals can also be measured by indirect calorimetry from CO_2 production, although with less precision (see Lawrence and Pearson 1989). Carbon dioxide entry rate, a measure of CO_2 production, may be measured by isotope dilution using ^{14}C-labelled sodium bicarbonate. Single injection (White and Leng 1969) or continuous infusion (Corbett et al. 1971) procedures are equally effective, but the former require multiple sampling of body fluids. In the continuous infusion procedure, the objective is to achieve constancy of SRA of $^{14}CO_2$ in blood, and for this reason, the infusion is continued for up to 12 hours (Whitelaw et al. 1972). The intrinsic problem with the method is that the CO_2 pool is not homogenous, but consists of many interlinked pools of varying turnover rate. Also, CO_2 is fixed in a number of metabolic cycles, and the recycling of labelled CO_2 is probably the major reason why even after 12 hours complete constancy of the SRA of $^{14}CO_2$ in expired air, blood, urine and saliva is not achieved. Nevertheless, the method is relatively simple and inexpensive, and has provided useful data on the energy expenditure of grazing animals (Corbett et al. 1971).

In general, the method is only applicable when the metabolic rate of the animal is relatively constant during the sampling period. With resting animals, Whitelaw et al. (1972) reported that values for CO_2 output obtained by isotope dilution differed by only 2-4% from values obtained by direct measurement of gas exchange. The procedure is inapplicable to working animals, where major changes in CO_2 pool size occur over short periods. Also, the nature of the system precludes its use to monitor responses over short time intervals.

Doubly-labelled water is also used to measure CO_2 production. The basis of this method is that whereas hydrogen is lost from the body mainly as water, O_2 is lost both in water and as CO_2 (Lifson et al. 1955). This implies that if the animal is given a dose of water enriched with $^2H_2{}^{18}O$ to label the total H_2O pool, the degree of enrichment (specific activity) of ^{18}O will decline faster than that of deuterium. Further, the difference in the two rates of decrease multiplied by the volume of the total H_2O pool is a measure of loss of CO_2. Total body water, incidentally, may be estimated from the specific activity of body water shortly after administration of the labelled water.

In practice, the specific activities of both deuterium (^2H) and ^{18}O are determined in any body fluid such as saliva or urine for up to 14 days following the administration of doubly-labelled water. Measurements are made during the first few hours to determine both the initial equilibrium specific activity, and total body water. In human subjects, measurements of specific activity are made after about 2 weeks, when the differences in the initial and final ratios of the specific activities of the two isotopes are large enough to be measured accurately.

The doubly-labelled water method has been compared with indirect calorimetry in studies with men (Seale et al. 1989). The authors observed reasonable agreement between energy expenditure determined using doubly-labelled water and that calculated from ME intake. These values, however, were much higher than those obtained by calorimetry. These differences were attributed to the higher levels of activity of the subjects when not physically restrained by the requirements of calorimetry.

The main advantage of the use of doubly-labelled water to measure energy expenditure is that the procedure is non-invasive, and requires only urine samples at infrequent intervals. The disadvantages are that the accurate determination of the isotopes requires analytical techniques based on mass spectrometry, and that the method gives only average data on total CO_2 production over time intervals of days. In studies on energy metabolism in humans pursuing normal activities, or in wild life studies where data are required on unrestrained animals, the doubly-labelled water procedure has considerable promise.

III. METABOLISM OF DEFINED TISSUES

(a) Arteriovenous difference measurements

The classical AV difference technique reached its full expression in the studies of the late Dr J.L. Linzell on the mammary gland of the lactating goat. Much of current knowledge of mammary gland metabolism, and in particular, the contribution of circulating metabolites to the synthesis of lactose, casein and milk fat synthesis is based on the efforts of Linzell and his many collaborators (see Annison 1983).

Zierler (1961) defined the stringent conditions that must be satisfied when the AV difference procedure is applied to the measurement of substrate uptake or release by defined tissues. These are that blood flow, tissue uptake of substrate and arterial concentration must all remain constant, and that venous blood must be representative of the total venous effluent of the tissue. Complete steady state conditions rarely apply, but in practice acceptable constancy of conditions is usually achievable.

The precision of AV difference measurements is directly related to the accuracy with which blood flow can be measured. The new procedures for the continuous measurement of blood flow based on ultrasound have solved problems stemming from the variability of blood flow which has adversely influenced earlier methods based on limited numbers of discreet measurements (Gooden and Giles 1991).

(b) Arteriovenous difference measurements combined with isotope dilution

Measurements of entry rate based on the continuous infusion into arterial blood of carbon-14 labelled substrate results in constancy of SRA of circulating substrate for a period of several hours. If AV difference measurements across defined tissues are made during this period, information is also obtained on the uptake, release and oxidation of the metabolite being studied (Linzell and Annison 1975). The procedure has been applied to a range of tissues.

(i) Mammary gland metabolism

Combined AV difference and isotope dilution procedures have provided comprehensive data on the uptake and utilization of glucose and acetate by the mammary gland, and on the rates of entry and oxidation of the same substrates in the whole animal (Linzell and Annison 1975).

The combined procedures proved particularly useful in the elucidation of the mechanism of uptake of circulating triglycerides by the mammary gland. Studies based on the intra-arterial infusion of chylomicrons containing triglycerides doubly-labelled with ^{14}C-glycerol and ^{3}H-palmitate showed that complete hydrolysis preceded the uptake of triglycerides by mammary tissue (West et al. 1972).

Isotope dilution combined with AV difference measurement has been used to measure rates of protein synthesis and degradation in the mammary gland of lactating goats (Oddy et al. 1988). Evidence was obtained of significant turnover of milk proteins, an unexpected finding which impinges on both protein and energy metabolism in mammary tissues.

(ii) Hind limb metabolism

The in vivo technique developed by Domanski et al. (1974) for the measurement of AV differences of circulating substrates by the hind limb may be combined with isotope dilution. The technique was modified by Teleni and Annison (1986) in an effort to sample venous drainage only from a defined muscle bed. The preparation was subsequently used to study valine metabolism, and rate of protein turnover was calculated from the data (Teleni et al. 1986).

Oddy and Lindsay (1986) have used the hind limb preparation to measure rates of protein synthesis and degradation in muscle in experiments based on the continuous infusion of ^{14}C-labelled leucine.

(iii)) Peripheral tissue metabolism

Harris and Lobley (1991) have developed a surgical preparation in sheep which permits the measurement of the metabolism of skin by AV difference and isotope dilution procedures. Application of the preparation has revealed that skin maintains a lower energy exchange but higher protein metabolism per unit mass than muscle.

IV. MEASUREMENT OF ABSORBED NUTRIENTS

An established procedure for the measurement of nutrient inflows into the portal circulation involves measurement of both portal-arterial (PA) difference, and blood flow. The application of this technique is best exemplified by the comprehensive studies of the late E.N. Bergman and his colleagues, which were reviewed by Bergman (1990). These studies, and most others have been based on the measurement of blood flow by dye dilution, which can only be applied at discrete intervals. The current availability of a robust and accurate method for the continuous measurement of blood flow based on ultrasound, however, has encouraged this laboratory to measure PA differences of a range of nutrients in sheep.

The ultrasonics procedure for the measurement of blood flow was compared with the conventional dye dilution procedure (Katz and Bergman 1969) under conditions designed to minimize potential interference to the precision of dye dilution (Lush et al. 1989). Mean values for jugular blood flow measured by the two methods were not significantly different. In contrast, direct comparison of the two methods for the measurement of portal flow in sheep (J.M. Lush and J.M. Gooden, unpublished) has shown that, at least in this laboratory, dye dilution overestimates portal blood flow.

The dye dilution method was used to measure blood flow in three sheep prepared for the continuous measurement of portal blood flow. Eight separate dye dilution measurements were made on each sheep and compared with mean values for portal blood flow recorded over the same time intervals. Mean values for portal blood flow measured by dye dilution and ultrasound were 97.9 ± 6.9 (s.e.) and 68.2 ± 3.7 ml/min/kg$^{0.75}$ respectively. The mean of the values for portal blood flow measured by dye dilution reported by Bergman and his colleagues (Bergman et al.

1970; Wolff et al. 1972) was 92.6 ± 13.7 ml/min/kg$^{0.75}$. We have no explanation for the higher values given by dye dilution, but laminar flow resulting in inadequate mixing of dye with blood at the sampling site would explain the widely variable data.

V. CONCLUDING COMMENTS

The range of procedures now available for the measurement of various facets of metabolism, many of which depend on the accurate measurement of blood flow, provide a valuable backdrop to studies of the factors which regulate metabolism. Rate of blood flow per se may have a major influence on tissue metabolism (see Black 1988); the increased milk yield in ruminants which occurs in response to growth hormone administration may be attributed in part to raised mammary blood flow (see McDowell and Annison 1991).

Many of the procedures discussed in this review are invasive, and it must be stressed that their use is justified only if data crucial to the success of the research cannot be obtained in any other way. Since even minor discomfort, irrespective of cause, may have significant physiological effects, minimization of stress in experimental animals is essential in quantitative nutritional studies. A promising non-invasive procedure for the measurement of metabolic parameters such as gluconeogenesis has been described which is based on nuclear magnetic resonance (Williams and Gadian 1986). At present the method is applicable only to small laboratory animals, but the approach has great promise for the future.

REFERENCES

ANNISON, E.F. (1983). In "Biochemistry of Lactation", p.399, ed T.B. Mepham. (Elsevier: Amsterdam).

ANNISON, E.F. (1984). In "Herbivore Nutrition", p.549, eds F.M.C. Gilchrist and R.I. Mackie. (The Science Press).

ANNISON, E.F., BROWN, R.E., LENG, R.A., LINDSAY, D.B. and WEST, C.E. (1967). Biochem. J. 104: 135.

ANNISON, E.F. and LENG, R.A. (1991). In "Proceedings of International Symposium on Nuclear and Related Techniques in Animal Production. (International Atomic Energy Agency), in press.

ANNISON, E.F., WHITE, R.R. (1961). Biochem. J. 80: 162.

ARMSTRONG, D.G. and BLAXTER, K.L. (1957). Br. J. Nutr. 11: 413.

BALLARD, F.J., HANSON, R.W., and KRONFELD, D.S. (1969). Fed. Proc. 28: 218.

BERGMAN, E.N. (1990). Physiol. Ref. 70: 567.

BERGMAN, E.N., KATZ, M.L. and KAUFMAN, C.F. (1970). Am. J. Physiol. 219: 785.

BLACK, J.L. (1988). J. Anim. Sci. (Suppl.3) 66: 22.

BLACK, J.L., GILL, M., BEEVER, D.E., THORNLEY, J.H.M. and OLDHAM, J.D. (1987). J. Nutr. 117: 105.

BLAXTER, K.L. (1962). 'The Energy Metabolism of Ruminants', (Hutchinson, London).

BLAXTER, K.L. (1989). 'Energy Metabolism in Animals and Man', (Cambridge University Press).

CORBETT, J.L., FARRELL, D.J., LENG, R.A., McCLYMONT, G.L., and YOUNG, B.A. (1971). Brit. J. Nutr. 26: 277.

DOMANSKI, A., LINDSAY, D.B. and SETCHELL, B.P. (1974). J. Physiol. Lond. 242: 28P.

GILES, L.R., GOODEN, J.M., TUCKER, R.G., ANNISON, E.F. and BLACK, J.L. (1989). In "Manipulating Pig Production", vol. III, p.207, eds. J.L. Barnett and D.P. Hennessey. (Australasian Pig Science Association).

GILES, L.R., GOODEN, J.M., LORSCHY, M.L., ANNISON, E.F. and BLACK, J.L. (1990). Proc. Nutr. Soc. Aust. 15: 41.
GOODEN, J.M. and GILES, R. (1991). Proc. Nutr. Soc. Aust. (in press).
HARRIS, P.M. and LOBLEY, G.E. (1991). In "Physiological Aspects of Digestion and Metabolism in Ruminants", p.201, eds. T. Tsuda, Y. Sasaki and R. Kawashima. (Academic Press).
LAWRENCE, P.R. and PEARSON, R.A. (1989). In "Draught Animals in Rural Development", ACIAR Proc. 27, p.155.
LIFSON, N., GORDON, G.V., and McCLINTOCK, R. (1955). J. Appl. Physiol. 7: 704.
LUSH, J.M., GOODEN, J.M. and ANNISON, E.F. (1989). Proc. Nutr. Soc. Aust. 14: 147.
KATZ, M.L. and BERGMAN, E.N. (1969). Am. J. vet. Res. 30: 655.
LIANG, J.B., TERADO, F. and HAMAGACHI, I. (1989). In "Energy Metabolism of Farm Animals", p.348. (EAAP Publication No.43).
LINZELL, J.L., and ANNISON, E.F. (1975). In "Digestion and Metabolism in the Ruminant", p.306, eds. I.W. McDonald, A.C.I. Warner. (University of New England Publishing Unit).
McCLEAN, J.A., and TOBIN, G. (1987). 'Animal and Human Calorimetry', (Cambridge University Press).
McDOWELL, G.H. and ANNISON, E.F. (1991). In "Physiological Aspects of Digestion and Metabolism in Ruminants", p.231, eds. T. Tsuda, Y. Sasaki and R. Kawashima. (Academic Press).
ODDY, V.H. and LINDSAY, D.B. (1986). Biochem. J. 233: 417.
ODDY, V.H., LINDSAY, D.B. and FLEET, I.R. (1988). J. Dairy Res. 55: 143.
PELL, J.M., CALDARONE, E.M. and BERGMAN, E.N. (1983). Biochem. J. 214: 1015.
PETHICK, D.W., and LINDSAY, D.B. (1982). Brit. J. Nutr. 48: 549.
SCHOENHEIMER, R., and RITTENBERG, D. (1935). Science 82: 156.
SCHOENHEIMER, R., RUTNER, S. and RITTENBERG, D. (1939). J. biol. Chem. 130: 730.
SEALE, J.L., RUMPLER, W.V. and CONWAY, J.M. (1989). In "Energy Metabolism of Farm Animals", p.337. (EAAP Publication No.43).
STEELE, R. (1964). Fed. Proc. 23: 671.
STEELE, R., WALL, J.S., de BODO, R.C. and ATSZULER, N. (1956). Amer. J. Physiol. 187: 15.
TELENI, E. and ANNISON, E.F. (1986). Aust. J. biol. Sci. 39: 271.
TELENI, E., ANNISON, E.F., and LINDSAY, D.B. (1986). Aust. J. biol. Sci. 39: 379.
WHITE, R.G., and LENG, R.A. (1969). Proc. Aust. Soc. Anim. Prod. 7: 335.
WHITELAW, F.G., BROCKWAY, J.M. and REID, R.S. (1972). Quart. J. exp. Physiol. 57: 37.
WEEKES, T.E.C. (1991). In "Physiological Aspects of Digestion and Metabolism in Ruminants", p.183, eds. T. Tsuda, Y. Sasaki and R. Kawashima. (Academic Press).
WEST, C.E., BICKERSTAFFE, R. and ANNISON, E.F. (1972). Biochem. J. 126: 477.
WILLIAMS, S.R. and GADIAN, D.R. (1986). Quart. J. exp. Physiol. 71: 335.
WOLFF, J.E., BERGMAN, E.N. and WILLIAMS, H.H. (1972). Am. J. Physiol. 223: 438.
ZIERLER, K.L. (1961). J.Clin. Invest. 40: 2111.
ZIERLER, K.L. (1976). Circ. Res. 38: 459.