Letters to the Editors

Errors in blood flow measurements

We have to report (to our considerable embarrassment) a systematic error which has occurred in a series of papers that have appeared in the *British Journal of Nutrition*. The relevant papers and faults are shown separately. The mistakes arose as a consequence of a calculation error in the determination of blood flow in muscle. The method used was originally described by Pappenheimer & Setchell (1972). The method depends on infusing tritiated water at an exponentially decreasing rate and measuring the area under the arterio–venous (A–V) difference curve, which is compared with the final value attained. In the method as originally described, for measuring brain blood flow, samples of blood were obtained at 1-min intervals and areas were obtained by arithmetically summing each minute's A–V value. When this method was transferred to study muscle, in which blood flow is much slower than in brain, sampling was made at 2-min intervals. It was assumed that the area would be obtained in the same way. What was in fact obtained was the flow in ml/g per 2 min: blood flow was thus just double what it should have been. Fortunately the mistake has not had a major effect on the conclusions we drew. The new values, however, are much more in line with those obtained elsewhere (see, for example, Oddy *et al.* 1981).

We should emphasize that the referees who examined our publications could not reasonably be expected to detect this error. Since muscle blood flow is highly variable the values could hardly be regarded as inherently unlikely. The mistake was discovered by a visitor to the laboratory, Mr V. H. Oddy, who routinely used the method with a different sampling time.

We offer our apologies to the Journal and to readers who may have been misled.

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Oddy, W. H., Brown, B. W. & Jones, A. W. (1981). *Australian Journal of Biological Science* **34**, 419–426.
Pappenheimer, J. R. & Setchell, B. P. (1972). *Journal of Physiology, London* **226**, 48–50P.

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Acetate supply and utilization by the tissues of sheep in vivo. By D. W. PETHICK, D. B. LINDSAY, P. J. BARKER and A. J. NORTHROP, vol. 46, (1981), no. 1.

p. 102, line 22: $y = -0{\cdot}038 + 0{\cdot}32$ should be changed to $y = 0{\cdot}16 - 0{\cdot}019x$.
p. 104, Table 2: all muscle blood flow values should be halved.
p. 105, Table 4: all values for muscle should be halved and the sums reduced correspondingly.

Acetate metabolism in lactating sheep. By D. W. PETHICK and D. B. LINDSAY, vol. 48, (1982), no. 2.

Only muscle blood flow is affected. Although the same technique was used for mammary flow, it happens that all the values reported in the paper relied on a sampling rate (1 ml/min) which would not have given rise to error in the estimation of blood flow.

p. 323, Table 2, net utilization: all values should be halved for glucose, L-lactate and D(−)-3-hydroxybutyrate.
p. 324, Fig. 2: ordinate values should be halved. All equations in the legend to the figure should thus be halved.
p. 325, line 2: values should be halved.
p. 326, Table 3, net muscle uptake: values should be halved.

Metabolism of ketone bodies in pregnant sheep. By D. W. PETHICK and D. B. LINDSAY, vol. 48, (1982), no. 3.

p. 557, Fig. 4: ordinate values should be halved. Values for V in legend should be halved.
p. 558, lines 16–18 up: for 'Ketone body formation was 1·3 mmol/h per kg muscle which represented 22% of the gross utilization of ketones by the muscle' read 'Ketone body formation was 0·65 mmol/h per kg muscle which represented 11% of the gross utilization of ketones by muscle'.
p. 560, Fig. 6 legend: mean blood flow should be 6·5 (SE 0·73) litres/h per kg muscle. All rate constants should be halved.
p. 560, 10 lines up: for '16%' read '8%'.

The metabolism of circulating non-esterified fatty acids by the whole animal, hind-limb muscle and uterus of pregnant ewes. By D. W. PETHICK, D. B. LINDSAY, P. J. BARKER and A. J. NORTHROP, vol. 49, (1983), no. 1.

p. 133, Fig. 1 legend: all values should be halved.
p. 134, Fig. 2: ordinate values should be halved. The slope and intercept of line of best fit should be halved.

Thyroid hormone assay

We wish to make two comments on the exchange of letters between Cox and Millward, and Lunn and Sawaya (*British Journal of Nutrition* (1985), **54**, 321–322).

The first relates to the reliability of the free T_3 assays used. The analogue-based free T_3 and free T_4 assays are supposedly based on analogues unbound by serum proteins. In reality, no such analogue has been identified (Ekins, 1984). None of the commercial kits conforms to the physico-chemical theory on which they are based, and all yield distorted results when the analogue-binding characteristics of test samples differ from those of the standards (Ekins, 1984; Jackson & Ekins, 1986). It is therefore predictable that the free hormone values they yield in rat serum will be in error (since human and rat serum binding proteins differ); also that apparent changes in free hormone concentrations when animals are treated in ways that change the protein composition of blood will be meaningless. Meanwhile Cox and Millward have (understandably) been misled into believing that the analogue used in the Diagnostic kit does not bind to thyroid-hormone-binding globulins. This is not true, and there are no grounds for supposing that analogue binding in rat serum is greater than that in human serum. Their finding of lower free T_3 results using the Diagnostic kit suggests the opposite in fact.

To summarize, in spite of manufacturers' claims, current labelled analogue methods do not measure free hormone concentrations in blood and they fail all tests of free hormone assay validity. Aside from their liability to error caused by differences in the protein content of test samples, the results they yield are also severely affected by binding 'competitors', e.g. free fatty acids (Ekins *et al.* 1983). We therefore strongly recommend that they should not be used in studies of the kind described in the above correspondence, nor in any research studies in which species or temporal differences in serum composition are likely.

Both analogue-based and Lepetit kits rely on the assumption that dissociation of hormone from serum binding proteins is not rate-limiting. There is, therefore, no reason to suppose that assay results with the Lepetit kit will be more affected by changes in the dissociation rate constant of the bound hormone complex. Lunn and Sawaya's belief that the Lepetit method is unique in depending on a physical separation of one component of a system in equilibrium is also invalid; the introduction of a (solid-phased) antibody into serum results in sequestration of free hormone in essentially the same manner as occurs in the Lepetit method. Thus, though no free hormone assay is faultless, there are strong theoretical and experimental reasons for supposing the Lepetit results to be the more reliable in studies of the kind reported.

The second comment is on the work of Pardridge & Mietus (1980). These authors did not, in fact, suggest that bound thyroid hormone enters hepatic cells in consequence of endocytosis. The concept underlying Pardridge's original hypothesis (Pardridge, 1981) is that bound hormone dissociates and enters target cells in tissues characterized by 'long' capillary transit times (i.e. transit times comparable with the half dissociation time of the bound hormone complex) but its mathematical basis has been shown to be invalid (Ekins *et al.* 1982). Pardridge & Landaw (1984) later abandoned it and the experimental data on which these ideas rest have frequently been criticized (e.g. Robbins & Johnson, 1982).

Pardridge & Landaw (1984) have now adopted a modification of the Kety–Renkin–Crone equation originally derived by Ekins *et al.* (1982) but which, in the simplified form relied on by these authors, disregards the rate limitations on hormone efflux from the capillary imposed by intracapillary hormone diffusion and protein bound hormone dissociation. Because their experimental data do not accord with this simple model, Pardridge *et al.* (1985) have hypothesized that local endothelially-derived factors present in the microcirculation affect the binding of hormone to binding proteins, and thereby facilitate hormone transport into certain tissues. Though we would disagree with this conclusion, valid physico-chemical reasons exist to support the view that changes in binding protein concentrations may affect the pattern of hormone transport in the body (Ekins *et al.* 1982). This possibility should be borne in mind in any consideration of the metabolic effects of alterations in thyroid hormone and binding protein levels resulting from changes in nutritional status.

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Ekins, R. P. (1984). *Journal of Clinical Immunoassay* **7**, 163–180.
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Pardridge, W. M. & Mietus, L. J. (1980). *Journal of Clinical Investigation* **66**, 367–374.
Pardridge, W. M., Premachandra, B. N. & Fiever, G. (1985). *American Journal of Physiology* **248** (*Gastrointestine Liver Physiology* 5), G545–G550.
Robbins, J. & Johnson, M. L. (1982). In *Free Hormones in Blood*, pp. 53–64 [A. Albertini and R. P. Ekins, editors]. Amsterdam, New York, Oxford: Elsevier Biomedical.

Energetic efficiency and amino acid supply in ruminants

A currently fashionable theory is that amino acid supply can influence the efficiency of utilization of metabolizable energy (ME) for fattening (k_f) in sheep given forage rations. In a recent paper, MacRae *et al.* (1985) attempted to explain the unusually low k_f of autumn grass on this basis and to find support for the theory. While the authors should be commended for addressing this interesting question, serious defects in the paper undermine most of their conclusions.

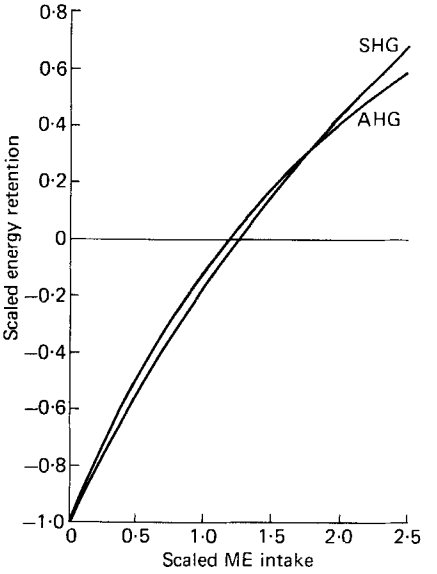
They measured energy balances with sheep given grass harvested from a mixed sward in the spring (SHG) and autumn (AHG) and examined the effect of abomasal infusion of casein on the energy value of AHG.

The paper does not mention that the k_f's of both feeds (0·54 for SHG and 0·43 for AHG), taken at their face value, agree well with predictions from ME concentration (0·54 and 0·40) using the Agricultural Research Council (1980) equation for first-cut forage. On this basis, AHG had a k_f commensurate with its digestibility which is not typical of autumn forages. Despite this quibble, the results could still provide a test of the theory about general variation of k_f but there are further problems.

The authors say: 'Although no significant difference exists between [exponential equations relating energy balance and metabolizable energy intake] for each grass, the curves ... are plotted in Fig. 1 to clarify the implications.' Unfortunately, the curve they drew for AHG does not match its equation or data and a false impression is created; the present figure offers an alternative view. If observations had been plotted, the error would have been discovered and the magnitude and range of the responses could have been taken into account by the reader; thus, display of even the mean results shows SHG and AHG to be similar as are AHG and AHG + casein.

Expt 2 is claimed to show that AHG supplied less amino-nitrogen per unit of ME than did SHG. However, this rests on results for the higher level of AHG that seem aberrant in the light of a few additional calculations. Thus, the digestibility of its non-ammonia N (NAN) in the small intestine (55%) and the contribution of amino-N to this (96%) are outside the range of values for all other treatments (65–68% and 83–87% respectively). Again, urinary N has a very high standard error in Expt 2 and the tabulated value for AHG at 1·5 times maintenance is about 3 g/d less than the difference between intake and faeces plus retention; even if corrected by this amount, this loss is outside the purview of the other values. If there is an explanation for these peculiarities, it should have been presented.

Finally, there were massive differences in live weight amongst the sheep (40–76 kg; 10–15 kg range within experiments) although they were all mature Suffolk × Greyface wethers, but no information was offered about the contribution of size, condition or fleece. This must have been one source of the excessive variation in certain balance elements which necessitated their correction to mean metabolic rate/$kg^{0·75}$ by covariance analysis (see footnote to Table 2). Since other elements were not adjusted, intake minus losses does not equate with retention in the tabulated balances, a serious matter which is not mentioned or discussed and whose impact cannot be assessed without additional information.



Relations between scaled energy retention and scaled metabolizable energy (ME) intake in sheep given spring-harvested (SHG) and autumn-harvested (AHG) dried grass calculated by the writer from the equations of MacRae *et al.* (1985).

The whole tenor of this paper is that an inferior k_f for autumn grass was demonstrated and that it was associated with an inferior capacity to supply amino acids, lending credence to a neat biochemical theory about the general control of k_f. I suggest that neither point is supported by the results. However, the work has been cited as conclusive both before (Anon, 1983) and since (Lobley & MacRae, 1986) its publication. Thus are myths created.

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Agricultural Research Council (1980). *The Nutrient Requirements of Ruminant Livestock*, no. 1. Slough: Commonwealth Agricultural Bureaux.

Anon (1983). *Rowett Research Institute, Annual Report of Studies in Animal Nutrition and Allied Sciences* **39**, 74

Lobley, G. E. & MacRae, J. C. (1986). *Proceedings of the 10th International Symposium on the Energy Metabolism of Farm Animals, Washington DC.* (In the Press).

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Reply to letter by Graham

Dr Graham's astute attention to detail has brought to our attention three arithmetic errors in our paper which were not identified either during its preparation or its subsequent editorial checking. We would like to correct these and apologise for the confusion this has caused. Our biological reasoning in the paper was based on analyses of our original data which we firmly believe support the comments made. We accept, however, that the errors identified below did not allow the concerned reader to follow this reasoning. We hope the following corrections will answer several of Dr Graham's misgivings.

1. *Energy balance data in Table* 2 (*paragraph* 6 *of the letter*). Our statistical author advised that in Expt 1 certain data in Table 2, namely urine energy excretion and heat production values, should be adjusted by covariance analysis to take account of the different fasting metabolic rates of the individual sheep (see footnote to Table 2). Unfortunately those adjustments altered the ME intake (MEI) and energy retention data but this was overlooked in the final preparation of the table. The final MEI and energy retention values should read:

	SHG(L)	SHG(H)	AHG(L)	AHG(H)
MEI (kJ/d)	8370	14767	7104	13204
Energy retention (kJ/d)	436	3751	−350	2421

2. *Urine-N data in Table* 3 (*paragraph* 5 *of the letter*). The urine-N excretions for all treatments are in error due to the incorrect input of one value for one sheep into analysis of variance. The correct values (g N/d) should read SHG(M) 6·6, SHG(1·5 M) 7·3, AHG(M) 8·6, AHG(1·5 M) 10·1. The next line should now read ΔN excreted in urine (g N/d) SHG 0·8, AHG 1·5. The N retention data are not altered.

3. *Mathematical representation of Fig.* 1. The B and p values for AHG are incorrectly reported on p. 201 of the paper due to an input error as above. They should read AHG $B = 2{\cdot}12$, $p = 0{\cdot}51$. These corrected values are now consistent with the corrected data for Table 2 and the original Fig. 1 which was based on the raw (i.e. untransposed) values. The figure in Dr Graham's letter includes the representation of the erroneous relations for AHG. Rather therefore than comment on his accusation that we exceeded our data by presenting Fig. 1, we simply apologise for the confusion caused.

We would also like to reply to other rather contentious points that Dr Graham has raised. (*a*) His observations on the N absorption data obtained in Expt 2 are obvious and sensible and were amongst the first we considered when analysing the results. The technique adopted to measure duodenal and ileal flow rates (Faichney, 1975) is, we believe, the best available at the present time and it has been successfully applied in many experimental situations. We have no reason to disbelieve the flow data. Indeed there are reasons for thinking that the ileal NAN flow rates might be higher on the AHG ration. The soluble carbohydrate content of AHG was only half that of SHG and so NAN entering the small intestine would probably have contained a higher proportion of dietary undegraded N and/or endogenous N. These fractions can be less well digested in the small intestines (see Siddons *et al.* 1985). (*b*) The question of the high proportion of the incremental NAN absorption on the AHG ration which could be accounted for as amino acids also bothered us but our amino acids analysis section is well equipped and authoritative and no anomalies could be found in the reported data. Dr Graham would note of course that although this aspect tended to reduce the difference between rations (whereas the between-diet differences in the extra NAN absorption per unit of MEI above maintenance was threefold, the differences in extra amino acid absorption per unit MEI was only twofold) we chose to concentrate on the amino acid data and thus err on the side of caution.

Finally, we hope that Dr Graham's comment on myth-building was not a serious accusation. The aim of science is to advance and develop hypotheses and then to test them. Our experiments were intended to do just that by examining whether the apparent differences in metabolic efficiency between two grasses harvested during different seasons could be explained in terms of differences in nutrient uptake from the gastrointestinal tract; the question of whether the AHG was a typical autumn harvested grass was not especially relevant in our view. We still believe that the data presented in the paper do support the earlier theory (MacRae & Lobley, 1982) that amino acid supply may influence efficiency of utilization of ME above maintenance on forage rations (generously referred to by Dr Graham as 'currently fashionable') although we have always said, and recently restated (MacRae & Lobley, 1986), that there are other possible explanations. What we would like to see are reports of experiments by Dr Graham or others to test whether this hypothesis or alternative ones explain the productive differences between various forages.

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