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# Undernutrition in sheep. Nitrogen repletion by N-depleted sheep

BY F. D. DEB. HOVELL, E. R. ØRSKOV, D. J. KYLE AND N. A. MACLEOD
*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

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1. Wether lambs of 29–44 kg live-weight, totally nourished by the infusion of volatile fatty acids (VFA) into the rumen and casein into the abomasum, were given five treatments in consecutive periods. The treatments were (daily amounts per kg live weight $(W)^{0.75}$): (a) high-protein for 7 d (2500 mg nitrogen, 650 kJ VFA); (b) low-protein for 7–15 d (525 mg N, 650 kJ VFA); (c) N-free for 7 d (no N, 450 kJ VFA); (d) very-low-protein for 24–28 d (300 mg N, 400 kJ VFA); (e) high-protein for 40 d (2500 mg N, 650 kJ VFA). Nine lambs were subjected to treatments (a), (b) and (c) (Expt 1) and four of the lambs additionally received treatments (d) and (e) (Expt 2).

2. In Expt 1 all nine lambs had a positive N retention on treatment (a) but abrupt change to treatment (b) resulted in substantial negative N balances initially, and a period of approximately 5 d adaptation was required before N equilibrium was re-established. Animals again exhibited negative N balances when the N-free infusion (treatment c) was introduced and during that period there was no evidence of adaptation. Basal urinary N excretion was estimated to be 356 (SE 12) mg N/kg $W^{0.75}$.

3. In Expt 2 all four lambs were depleted of N when receiving the very-low-protein treatment (d). The progressively decreasing N losses recorded during days 1 to 12 of the treatment period were slightly greater than those recorded during days 13 to 28 but the difference between the means was not significant ($P > 0.05$). There was no evidence of an adaptation in N retention between days 13 and 28 of the treatment. As assessed during days 13 to 28 of the treatment the efficiency of utilization of infused casein N was 1·0; this compared with a value of 0·66 recorded during treatment (b) in Expt 1. Live weight loss during the period of N depletion was 101 (SE 27) g/d.

4. When lambs were given treatment (e) during the last period of Expt 2, N repletion was rapid and complete within a few days. Ten days after the introduction of the treatment the rate of N retention was estimated to be 1019 (SE 38) mg/kg $W^{0.75}$ per d and this value declined at a rate of 9·5 (SE 1·9) mg N/kg $W^{0.75}$ per d for the following 30 d. In comparison, N retention determined for the high-protein treatment in Expt 1 was 724 (SE 66) mg N/kg $W^{0.75}$ per d. Live-weight gains during N repletion were 292 (SE 26) g/d.

5. It is concluded that N-depleted lambs can replete rapidly and that enhanced N accretion (compensatory growth) may persist for 4–5 weeks. If the improved efficiency of utilization of infused N observed during N depletion reflects a changed basal N requirement, the validity of simple factorial systems for estimating N requirement is called into question.

Because of the link between digestible energy intake and microbial protein synthesis in the rumen, energy undernutrition in ruminant animals is normally accompanied by protein undernutrition, defined in terms of the supply of amino acids to the small intestine. The development of a method for the complete nourishment of ruminants by intragastric infusion (Ørskov *et al.* 1979) has allowed energy and protein nutrition to be varied independently, and has enabled studies of basal nitrogen excretion to be undertaken. These studies (Hovell *et al.* 1983*a*, *b*; Ørskov *et al.* 1983) have shown that in animals receiving diets providing energy intakes much below the maintenance requirement, microbial N supply from the rumen would not be sufficient to meet the animal's basal N losses, and this must lead to a progressive N depletion. Such depletion occurs in practice in systems of animal production which involve a seasonal period of restricted food supply. However, the losses of energy and protein are generally made good in subsequent periods when food is plentiful and at such times animals may exhibit accelerated or compensatory growth (Wilson & Osbourn, 1960; Allden, 1970; Ørskov *et al.* 1976; Reid & White, 1977).

The main objectives of the experiment reported here were to study the potential of N depleted animals to make good protein losses and to investigate whether any subsequent accelerated rates of protein accretion were simply repletion or had characteristics of compensatory growth. A further objective was to study whether any adaptation occurred during a period of low-protein nourishment. Additional measurements of basal urinary N excretion (UN(E)) were also made.

## EXPERIMENTAL

### *Animals*

Nine wether lambs of between 30 and 42 kg and 4–6 months of age were used. They had each been fitted with a permanent rumen cannula of about 18 mm external diameter and a permanent abomasal catheter of about 5 mm external (3 mm internal) diameter. The lambs were weighed initially, after they had been adapted to infusion, and at intervals during the experiment. All calculations were made on the basis of the initial weight.

### *Housing and management*

The lambs were kept in metabolism cages fitted with an expanded-metal, PVC-coated floor. The volatile fatty acids (VFA) and buffer concentrate solutions and casein solution were prepared as described by MacLeod *et al.* (1982), and diluted as appropriate before infusion. The VFA solution contained acetic, propionic and butyric acids in molar proportions 0·65:0·25:0·10. The lambs were infused continuously, the volumes infused being kept constant, and the amounts of VFA, buffer and casein infused varied by changing the concentration of the infusate. About 200–300 g diluted casein solution/kg live weight $(W)^{0.75}$ were infused daily into the abomasum. VFA and buffer solutions were infused into the reticulo-rumen with total amounts being about 0·8–1·0 kg/kg $W^{0.75}$ per d. One-third of this was VFA solution and two-thirds buffer. These volumes are larger than those described by Ørskov *et al.* (1979) and MacLeod *et al.* (1982) and more in accord with those of Hovell *et al.* (1983*b*). The objective in infusing these larger volumes was to give better control of rumen pH and osmotic pressure (OP), to keep rumen OP below 350 mosmol/l and preferably below 300 mosmol/l and to keep rumen pH above 5·2 and preferably above 5·8. Departure from these limits of OP and pH usually necessitates that the rate of VFA infusion be reduced. The reason for this appears to be that the rate of VFA absorption is related to the rate of water absorption (Engelhardt & Hauffe, 1975) which in turn is defined by rumen OP (Engelhardt, 1970). Engelhardt (1970) cites work from his laboratory which demonstrated a reduction in water absorption when OP rose above about 325 mosmol/l. Unpublished work from our own laboratory (N. A. MacLeod, personal communication) has shown that at an OP of about 400 mosmol/l, the rate of VFA absorption falls below that of VFA infusion, and that VFA absorption rate tends to be less at an OP of 360 mosmol/l than at an OP of 320 mosmol/l. The amount of buffer infused was between 0·2 and 0·4 mol bicarbonate (0·3 potassium:0·7 sodium)/mol VFA. The actual amount of buffer concentrate infused, which varied between animals, was kept as low as possible consistent with maintaining rumen pH. All minerals, trace elements and vitamins were included in the infusates or dosed into the animals as described by MacLeod *et al.* (1982).

### *Collection of excreta and chemical analysis*

Urine was collected daily in sulphuric acid and analysed for N and creatinine as described by Hovell *et al.* (1983*b*). Care was taken that all urine samples were below pH 4·0 to ensure no conversion of creatinine to creatine.

### *Treatments and design*

Following adaptation to nourishment by total infusion, the levels of VFA and casein infused were increased gradually until VFA energy was equivalent to 650 kJ/kg $W^{0.75}$ and casein-N to 2500 mg/kg $W^{0.75}$ daily. The treatments shown in Table 1 were then imposed in sequence.

Table 1. *Sequence of treatments imposed (planned levels of infusion)*

| Treatment | Infusions (/kg $W^{0.75}$ per d) VFA (kJ) | Casein-nitrogen (mg) | Duration (d) |
|---|---|---|---|
| (a) High-protein | 650 | 2500 | 7 |
| (b) Low-protein | 650 | 525 | 7–15 |
| (c) Nitrogen-free (depletion) | 450 | nil | 7 |
| (d) Very-low-protein (depletion) | 400 | 300 | 24–28 |
| (e) High-protein | 650 | 2500 | 40 |

W, live weight; VFA, volatile fatty acids, infused in the molar proportions 0·65 acetic:0·25 propionic:0·10 butyric.

Changes from high-protein (treatment (a)), to low-protein (treatment (b)), to N-free (treatment (c)) to very-low-protein (treatment (d)) were made abruptly. The change from very-low-protein (treatment (d)) back to high-protein (repletion; treatment (e)) was made over a period of approximately 1 week. All nine animals were subjected to the first three treatments (Expt 1) in which UN(E) was measured. Four of the animals were also subjected to N depletion and subsequent repletion (Expt 2). The level of nutrition chosen for the period of depletion was set such that the animals were at approximate energy equilibrium but in slight protein deficit. The ratio, casein nitrogen:energy (750 mg N/MJ) was slightly less than that suggested by Agricultural Research Council (1984) to be supplied by microbial N (911 mg truly digestible amino acid N/MJ metabolizable energy).

### *Statistical analysis*

Results are presented as means with standard errors calculated from individual animal means of *n* days observations. Regressions were fitted to the daily observations on individual animals. Slopes were combined where appropriate.

## RESULTS

*Animal health and management of infusion.* Animal health remained good and there were no problems attributable to the infusion technique or to N depletion in Expt 2. Repletion was initiated gradually, the high level of VFA and casein infusion being re-established within 5–9 d. The rate at which this was done was based on a subjective assessment of animal health, and on the stability of rumen pH and osmotic pressure, which in our experience provides an indication of good health in ruminants nourished by intragastric infusions. Once infusions were re-established at the high level, repletion was rapid with the lambs maintaining high growth rates for 40 d. All four lambs which had been depleted were successfully repleted.

### *Expt 1. Measurement of UN(E)*

*N retention before N-free infusion.* The mean daily N retention of nine lambs is shown in Fig. 1. Changing from the high- to the low-protein level had a dramatic effect on N retention, and equilibrium was not achieved until 5 or 6 d after the change in level. This was observed with the first two lambs investigated, and thereafter this period was extended to 10 d (five lambs) or 15 d (two lambs). There was no evidence of any further adaptation after

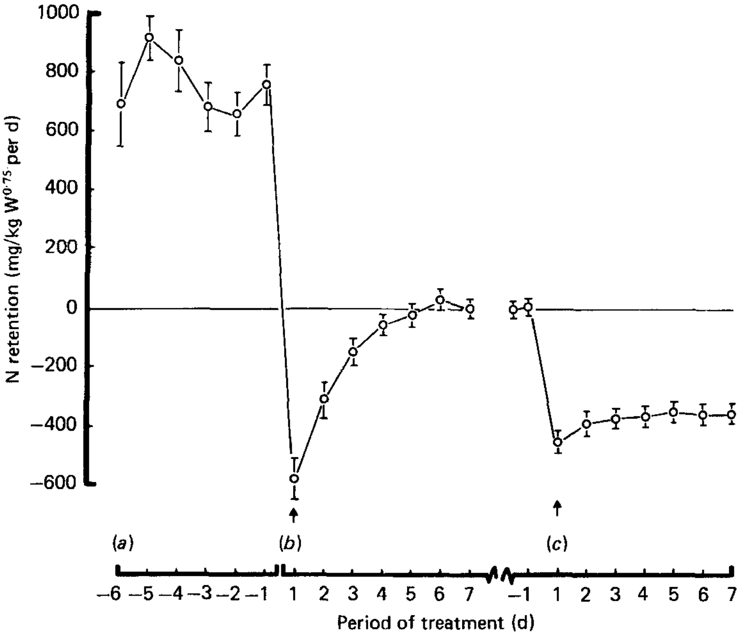

Fig. 1. Expt 1. Mean daily nitrogen retention of nine wether lambs of 29–44 kg live-weight (W) given 650 kJ/kg $W^{0·75}$ per d as volatile fatty acids (VFA) and 2500 (treatment (a)) or 525 (treatment (b)) mg casein-N/kg $W^{0·75}$ per d (high- and low-protein), or 450 kJ/kg $W^{0·75}$ per d as VFA and no protein (N-free, treatment (c)) in three consecutive periods. ↑, Change of treatment. Standard errors are represented by vertical bars.

Table 2. *Expt 1. Urinary excretion of nitrogen (UN(E)) and creatinine of wether lambs given 450 kJ/kg live weight (W)$^{0·75}$ per d as VFA and no protein for 7 d (treatment (c))*

(Mean values for days 3–7)

| Lamb | W (kg) | UN(E) (mg/kg $W^{0·75}$ per d) | Creatinine (mg/kg $W^{0·75}$ per d) |
|---|---|---|---|
| A | 43·7 | 326 | 50·4 |
| B | 30·6 | 335 | 51·7 |
| C | 29·3 | 387 | 48·4 |
| D | 29·3 | 304 | 46·7 |
| E | 32·1 | 401 | 54·7 |
| F | 30·3 | 386 | 47·2 |
| G | 39·0 | 384 | 50·0 |
| H | 39·0 | 318 | 49·1 |
| I | 29·3 | 367 | 45·2 |
| Mean | 33·6 | 356 | 49·3 |
| SE | 1·8 | 12 | 0·95 |

approximately 5 d. Thus the mean N retention of the seven lambs maintained at the low-protein level for an extended period was −34, +8, −17, −22, −8, −31 mg N/kg $W^{0·75}$ (SEM 16) for day 5 through to day 10 of the period. Nor was there any evidence that the length of this period was correlated with N loss during the subsequent period of N-free infusion.

Table 3. *Expt 2. Initial and final live weights before and after nitrogen depletion (treatments (c) and (d) Table 1), and after 40 d N repletion (treatment (e) Table 1)*

| | Live wt (kg) | | | Rate of live wt change (g/d) | |
|---|---|---|---|---|---|
| Lamb | Initial | Depleted | Repleted | Depletion | Repletion |
| A | 43·7 | 38·8 | 51·6 | −153 | 321 |
| B | 30·6 | 26·5 | 35·4 | −141 | 222 |
| C | 29·3 | 28·1 | 41·8 | −43 | 342 |
| D | 29·3 | 27·5 | 38·8 | −68 | 283 |
| Mean | 33·2 | 30·2 | 41·9 | −101 | 292 |

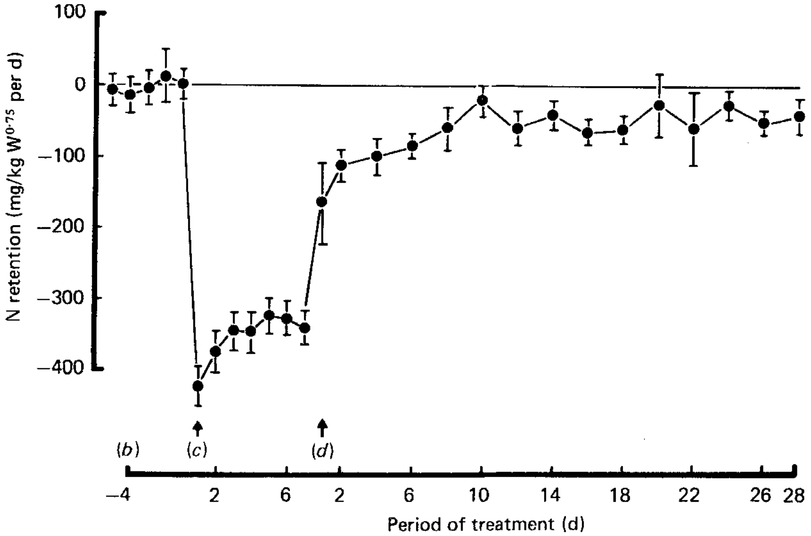

Fig. 2. Expt 2. Mean daily nitrogen retention of four wether lambs of 29–44 kg live-weight (W) given 650 kJ/kg $W^{0·75}$ per d as volatile fatty acids (VFA) and 525 mg casein-N/kg $W^{0·75}$ per d (low-protein, treatment (b)), 450 kJ/kg $W^{0·75}$ per d as VFA and no protein (N-free, treatment (c)) or 400 kJ/kg $W^{0·75}$ per d as VFA and 300 mg casein-N/kg $W^{0·75}$ per d (very-low-protein, treatment (d)) in three consecutive periods. ↑, Change of treatment. Values for treatment (d) from days 4–28 are averages of 2-d observations. Standard errors are represented by vertical bars.

*Endogenous N loss.* The mean UN(E) during days 3–7 and of the period of N-free infusion are given for the individual lambs in Table 2. There was no evidence of any adaptation during the period as is shown by Fig. 1.

### *Expt 2. Depletion and repletion*

*Live-weight changes.* Initial weights and weights at the end of depletion and after 40 d repletion are shown by Table 3. The lambs averaged about 100 g loss/d during depletion, and gained nearly 300 g/d during repletion.

*N depletion.* Body N losses for the four lambs depleted (lambs A, B, C and D) are summarized in Fig. 2. The mean cumulative loss of the four lambs was 5·58 g N/kg $W^{0·75}$ (SE 0·92), 75·2 g N in total (SE 9·6). Total losses were comparable with the body N losses

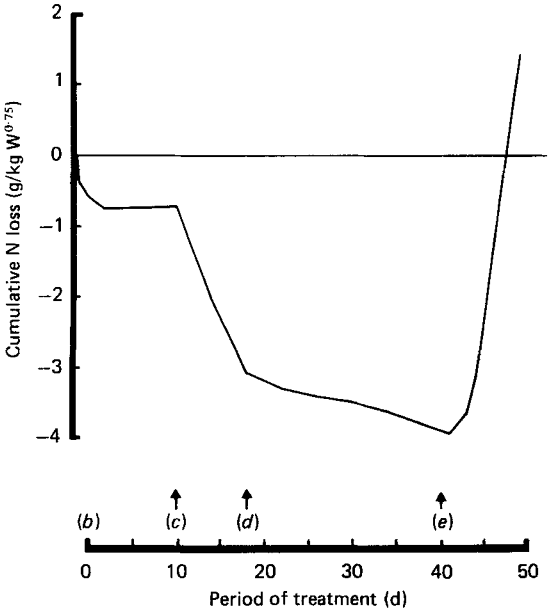

Fig. 3. Expt 2. Cumulative nitrogen loss (g/kg live weight $(W)^{0\cdot75}$) of a single wether lamb (lamb A) during three periods (low-protein, treatment (b); N-free, treatment (c); very-low-protein, treatment (d)) (for details, see Table 1), and N gain during the first few days of N-repletion (treatment (e)). ↑, Change of treatment.

Table 4. *Expt 2. Daily nitrogen balance and amounts of volatile fatty acids (VFA) and casein infused before and during N depletion of four wether lambs infused with low levels of protein (calculated on initial weight)*

(Mean values with their standard errors)

| | Mean | SE |
|---|---|---|
| Metabolic body-wt (kg live wt $(W)^{0\cdot75}$) | 13·8 | 1·07 |
| Before N depletion (treatment (b)) | | |
| VFA infused (kJ/kg $W^{0\cdot75}$) | 638 | 10 |
| Casein-N infused (mg/kg $W^{0\cdot75}$) | 518 | 19 |
| N retained (mg/kg $W^{0\cdot75}$) | 2 | 17 |
| Efficiency of N utilization* | 0·66 | 0·02 |
| Days 2–6 of depletion (treatment (d)) | | |
| VFA infused (kJ/kg $W^{0\cdot75}$) | 400 | — |
| Casein-N infused (mg/kg $W^{0\cdot75}$) | 297 | 4 |
| N retained (mg/kg $W^{0\cdot75}$) | −96 | 24 |
| Efficiency of N utilization* | 0·82 | 0·11 |
| Day 13 to end of depletion (treatment (d)) | | |
| VFA infused (kJ/kg $W^{0\cdot75}$) | 400 | — |
| Casein-N infused (mg/kg $W^{0\cdot75}$) | 300 | 1 |
| N retained (mg/kg $W^{0\cdot75}$) | −39 | 9 |
| Efficiency of N utilization* | 1·00 | 0·06 |

* Mean of days 5–10 (lamb B, 3 d only); for details, see p. 83.

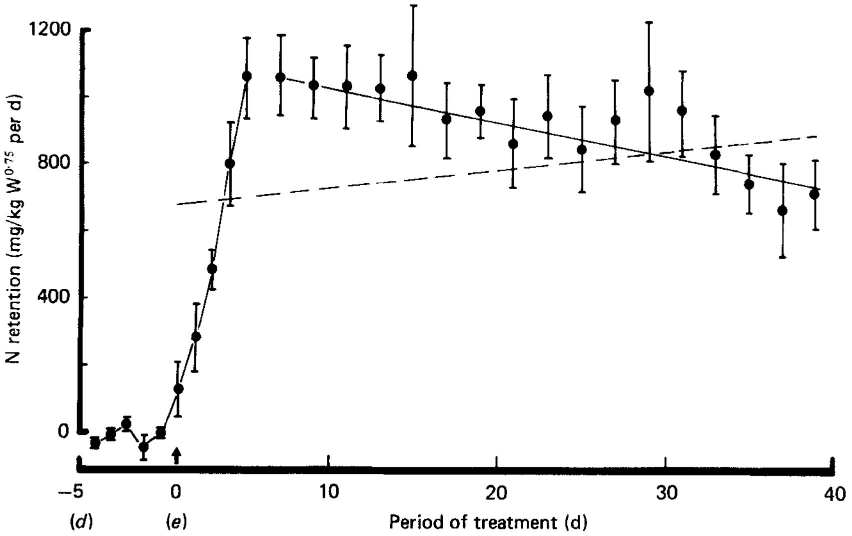

Fig. 4. Expt 2. Mean daily nitrogen retention calculated on initial weight of four wether lambs when infused with 400 kJ/kg live weight $(W)^{0.75}$ per d as volatile fatty acids (VFA) and 300 mg casein-N/kg $W^{0.75}$ per d (very-low-protein, treatment (d)) or 650 kJ/kg $W^{0.75}$ per d as VFA and 2500 mg casein-N/kg $W^{0.75}$ per d (N-repletion, treatment (e)). ↑, Change of treatment; (---), N retention at similar levels of infusion before depletion (treatment (a)) (corrected for W, see p. 84). Standard errors are represented by vertical bars.

of the normally fed sheep of Fattet *et al.* (1984) (75·2 g in 92 d). The typical pattern of the cumulative losses during the periods of N-free infusion and very-low-protein (comprising the depletion phase) for an individual lamb is shown in Fig. 3 (lamb A). The daily losses of body N over the period of very-low-protein infusion (treatment (d)) were examined with the objective of finding statistical evidence of adaptation. Various exponential and curvilinear models were tried, but the data were most accurately described by a non-linear loss until day 13 of the period and a linear loss thereafter. Only in one lamb did the slope of this regression approach statistical significance ($0{\cdot}10 < P > 0{\cdot}05$; a positive value implies a decreasing N loss with time and hence adaptation). The slopes did not differ significantly and the combined slope of 0·9 (SE 0·9) mg/kg $W^{0.75}$ provides no evidence of adaptation from day 13 of the depletion (treatment (d)). A comparison was also made of the efficiency with which infused N was utilized before depletion and during depletion (Table 4). (Efficiency was calculated as the change in N retention in response to the infusion of casein N, using the excretion of N during N-free infusion as the base value.) The mean apparent efficiency of utilization before depletion was 0·66 (SE 0·02) and during depletion (day 13 to the end) was 1·00 (SE 0·05), which implies adaptation between days 1 and 12 of the depletion phase with the very-low-protein infusion (treatment (d)).

*N repletion.* The average rate of N repletion is shown in Fig. 4, and a typical example of the pattern of the cumulative gain during the first few days in Fig. 3 (lamb A). Repletion was very rapid and was complete in about 1 week. In two of the four lambs, repletion was complete before the maximum level of energy as VFA (650 kJ/kg $W^{0.75}$ per d) and protein (2500 mg N/kg $W^{0.75}$ per d) infusion had been achieved. The rate of N retention peaked about 4–6 d after repletion started and declined thereafter. The rate of N retention decreased with time, the best description being a linear regression. In two of the four lambs the slope of this regression was significant ($P < 0{\cdot}01$). The individual slopes did not differ significantly, the combined slope being −9·5 (SE 1·9) mg N/kg $W^{0.75}$ per d. However, the

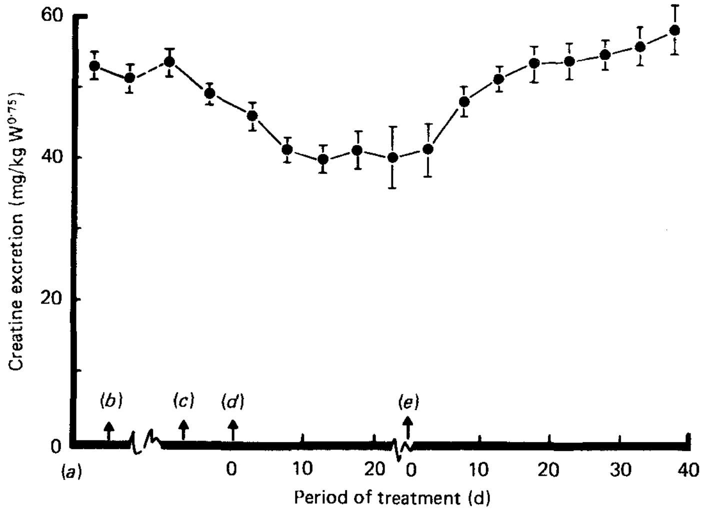

Fig. 5. Expt 2. Mean daily creatinine excretion (mg/kg live weight $(W)^{0.75}$ per d expressed as 5 d means) of four wether lambs during consecutive periods of volatile fatty acid (VFA) and casein infusion: high-protein (treatment (a)), low-protein (treatment (b)), N-free (treatment (c)), very-low-protein (treatment (d)), and high-protein (treatment (e)) as described in Table 1. ↑, Change of treatment. Standard errors are represented by vertical bars.

rate of N accretion continued to be greater than that achieved before depletion, for about 4–5 weeks. Since the animals were gaining rapidly in weight during this period, and the results as presented are based on weight at the start of the experiment, the results could be biased. Therefore a predicted accretion rate was calculated as the total N retention to be expected from the weight of the animal at any time during repletion (assuming linear growth), but also assuming the same N accretion rate per kg $W^{0.75}$ as that measured before depletion (when given the high protein infusion (treatment (a)). Comparison of measured accretion rates with the predicted accretion rates calculated in this way still demonstrated a compensatory effect which persisted to about week 4 (Fig. 4).

### *Creatinine excretion (Expts 1 and 2)*

The creatinine excretion during the period of N-free infusion (treatment (c), Expt 1) is given in Table 2, and the average excretion over the whole of Expt 2 of the four lambs depleted and repleted is shown in Fig. 5. The rate of creatinine excretion showed clear trends during the course of the experiment. It was reduced during the period of very-low-protein and energy infusion (treatment (d)) and subsequently increased during the repletion phase of high-protein and energy infusion (treatment (e)). There was no correlation between N and creatine excretion during the N-free period (treatment (c)). The mean daily creatinine excretion during this period was (Table 2) 49·3 (SE 0·95) mg/kg $W^{0.75}$ which was less ($P < 0.01$) than that of 53·6 (SE 1·0) and 52·4 (SE 1·57) mg/kg $W^{0.75}$ in the preceding periods of high- and low-protein infusion (treatments (a) and (b)) respectively.

## DISCUSSION

### *Creatinine excretion*

During N and energy restrictions in Expt 2, the changes in creatinine excretion were greater than could be attributed to conceivable losses in lean-body mass. Thus the mean daily

Table 5. *Expt 2. Daily nitrogen balance and amounts of volatile fatty acids (VFA) and casein infused during N repletion of four wether lambs infused with high levels of protein (calculated on initial weight)*

(Mean values with their standard errors)

| | Mean | SE |
|---|---|---|
| Metabolic body-wt (kg live wt $(W)^{0\cdot75}$) | | |
| Initial | 13·8 | 1·07 |
| After N depletion | 12·8 | 0·89 |
| After N repletion | 16·4 | 1·03 |
| Before N depletion* (treatment (a)) | | |
| VFA infused (kJ/kg $W^{0\cdot75}$) | 650 | 1 |
| Casein-N infused (mg/kg $W^{0\cdot75}$) | 2430 | 29 |
| N retained (mg/kg $W^{0\cdot75}$) | 724 | 66 |
| Repletion (treatment (e)) | | |
| VFA infused (kJ/kg $W^{0\cdot75}$)† | 657 | 7 |
| Casein-N infused (mg/kg $W^{0\cdot75}$)† | 2430 | 16 |
| N retained (mg/kg $W^{0\cdot75}$) | 1103 | 159 |
| Adjusted N retention (mg/kg $W^{0\cdot75}$)‡ | | |
| Day 10 | 1019 | |
| Day 20 | 924 | 107 |
| Day 40 | 734 | |

* Mean of 5 d.

† Calculated on first 2 d at full level of casein and VFA infusion.

‡ Calculated from combined slope of $-9\cdot5$ mg N/kg $W^{0\cdot75}$ per d (SE 1·9) and individual intercepts.

creatinine excretion for lambs A, B, C and D for the 5 d preceding the N-free period was 53·6 (SE 0·8) mg/kg $W^{0\cdot75}$ and for days 5–10 of the depletion phase was 41·1 (SE 0·5) mg/kg $W^{0\cdot75}$, a decline of over 23%. The mean cumulative N loss to this stage was 4·45 g/kg $W^{0\cdot75}$ which is equivalent to about 380 g protein in total, approximately 9% of total body protein (as calculated from Agricultural Research Council (1980)). Similarly, comparison of Fig. 5 with Fig. 3 shows that, although changes in creatinine excretion tended to move in the same direction as changes in N balance, the relation was not close. We have reported previously (Hovell *et al.* 1983*b*) that creatinine excretion fluctuates with the amount of energy infused. Therefore creatinine excretion cannot be used to estimate lean-body mass, or changes in the lean-body mass of sheep with acceptable precision.

### *Basal N excretion*

The UN(E) of 356 (SE 12) mg/kg $W^{0\cdot75}$ per d (Table 2) conforms well with that of 350 mg/kg $W^{0\cdot75}$ per d adopted by the Agricultural Research Council (1984). It is less than that of 429 (SE 21) mg/kg $W^{0\cdot75}$ per d for five lambs previously reported by ourselves (Hovell *et al.* 1983*a*). However, it agrees with the value of 356 mg/kg $W^{0\cdot75}$ per d for the average of the total of the lambs measured in our laboratory to that date and also described by Hovell *et al.* (1983*a*). Thus the value for UN(E) of 356 mg N/kg $W^{0\cdot75}$ per d now represents the mean of twenty-three lambs of between 26 and 44 kg live-weight. As shown in Table 2, individual animals vary considerably. Within the weight range observed, there was no obvious relation between live weight and N loss (based on kg $W^{0\cdot75}$). Analysis of the variance of the forty-five observations (nine animals for 5 d) showed the between-animal variation to account for 0·74 of the mean variance, and between-day variation for 0·09 of the mean variance. The residual standard deviation was 39 mg N/kg $W^{0.75}$ per d, equivalent to 0·11 of the overall mean (356 mg N/kg $W^{0.75}$ per d).

The volumes of urine produced by lambs on N-free infusion are more than 8 litres/d. Urination occurs frequently, and therefore end-of-period errors are unlikely to account for the day-to-day variation observed. If all variation not attributable to animals is included, basal excretion varied from day-to-day with a standard deviation of 48 mg N/kg $W^{0.75}$ per d. This gives a coefficient of variation of 0·13 which is less than that of 0·20 for endogenous N excretion of man (Food and Agriculture Organization/World Health Organization, 1973 cited by Waterlow *et al.* 1978). Whether this represents a true variation in metabolism or simply changes in the body urea pool has yet to be established.

### *N retention before depletion*

In infusion experiments in which the changes in the amounts of energy (Hovell *et al.* 1983*b*) or protein (Storm *et al.* 1983) have been relatively small (about 100 kJ or 100 mg N/kg $W^{0.75}$ per d), it has been our experience that stability in N retention is reached rapidly. Indeed, the 1st day at a new level is usually indistinguishable from the 5th day, although our normal practice is to discard the results of the first day. The large changes made in the experiments reported here in the amount of protein infused (from about 2500 down to 525 mg casein N/kg $W^{0.75}$ per d) showed that 5–6 d were required for stability in N retention to be achieved.

The average N loss by the nine lambs of Expt 1 was 581 (SE 66) and 307 (SE 62) mg/kg $W^{0.75}$ per d during the first 2 d of low-protein infusion (treatment (b)) when 525 mg casein N/kg $W^{0.75}$ per d were infused. Thus N excretion was (on the 1st day) more than double that infused. Experiments in our laboratory, also with infused sheep (F. G. Whitelaw, personal communication), have shown that the body urea-N pool was reduced by about 180 mg N/kg $W^{0.75}$ when casein N infusion was reduced from 1600 to 200 mg N/kg $W^{0.75}$ per d. This would suggest that only part of the change in N excretion observed in Expt 1 reported here was due to an adjustment of the body urea pool. However, this factor would have to be excluded before any firm conclusions could be made about short-term changes in rates of body protein accretion and depletion following large and rapid changes in protein supply.

### *Body-N losses during depletion*

Although there was no evidence of adaptation during the long term of the protein-depletion phase, the results suggest (Fig. 2, Table 4) that some degree of adaptation took place during the first few days of depletion. The change in the efficiency with which the casein-N appeared to be utilized (Table 4), could be interpreted as a change in basal (maintenance) requirement. A change of about 30% in UN(E) would be required to accommodate such a change in apparent efficiency. If this interpretation is correct, then the implication is that simple factorial estimates of protein requirements become invalid when animals are at a low level of nutrition. Some evidence of a change in basal N requirement is provided by the comparative slaughter experiment of Fattet *et al.* (1984). In that experiment, sheep given low levels of nutrition lost less body protein than would have been predicted on the basis of the Agricultural Research Council (1984) factorial estimates of N requirement. However, it should be remembered that calculations made for normally-fed ruminants have the added difficulty of possible variations in microbial protein supply.

The fact that we have been unable to detect an adaptation effect in N retention in animals given N-free infusions either in the experiment reported here (Fig. 1) or experiments reported previously (Hovell *et al.* 1983*a*) suggests that if adaptation does occur, it occurs only when there is a supply of exogenous amino acids. This could be due to a change in the dynamics of protein turnover. Golden *et al.* (1977) reported that with children recovering from malnutrition, the protein fractional synthesis rate was about three times more responsive to changes in energy supply than the fractional breakdown rate, and similar results have been obtained with rats (Millward *et al.* 1975). In the rat experiments, intakes of protein and energy were confounded. In the experiment reported here, the change from the N-free treatment to very-low-protein treatment (depletion phase) was made with a small reduction in the amount of VFA infused (changed from 400 to 450 kJ/kg $W^{0.75}$ per d, Table 1), approximately equal to the gross energy of the casein infused (changed from zero to 300 mg N/kg $W^{0.75}$ per d, Table 1).

In practice, the response of an animal is likely to depend not only on its current nutrient supply, but also on its nutritional history and stage of development. Waterlow *et al.* (1978) made this point, and our own experience with infused (Hovell *et al.* 1983*a*) and normally fed (Fattet *et al.* 1984) sheep provides evidence that the nutritional status of the animal at the time is important.

### *N retention during repletion*

The very rapid repletion of the lambs demonstrated their capacity to take immediate advantage of the improvement in protein supply. The enhanced rate of protein accretion continued until well after repletion was complete (Fig. 4). This type of compensation in normally fed animals is well documented as compensatory growth (Wilson & Osbourn, 1960; Allden, 1970; Ørskov *et al.* 1976; Reid & White, 1977). That compensation involves more than a simple repletion of tissues was also shown by Millward *et al.* (1975) who refed rats which had been given a protein-free diet for 30 d. A sevenfold increase in the fractional synthesis rate of protein was measured by the 8th day of re-alimentation, while the fractional breakdown rate of protein was increased by only twofold. These rates had shown only a slight decline by the end of the experiment at 14 d. We have shown with infused animals (Hovell *et al.* 1983*b*) that compensation may be expressed as an improved efficiency of N accretion following a period of energy restriction.

### *Practical implications*

The main practical implications of the experiments reported here are twofold. First, that N repletion following a period of N depletion can be very rapid, provided the supply of protein is adequate. Second, that if the changes in the apparent efficiency with which casein was utilized (when infused at the low levels) were due to real changes in the apparent basal N requirement, then the use of simple factorial systems for the estimation of requirements is called into question.

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