Contribution of liver, skin and skeletal muscle to whole-body protein synthesis in the young lamb

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1. Protein fractional synthesis rate (FSR) was measured in some major tissues and in the whole body of six 1-week-old sucking lambs by a large injection of L-[^{3}H]valine.

2. Upper estimates of tissue protein FSR (%/d), assuming that the tissue-homogenate free-valine specific radioactivity defined that of valyl tRNA, were 115·0 in liver, 24·1 in skin, 22·9 in the white M. tensor fasciae latae, 21·6 in the red M. diaphragma and 19·6 in the remainder (exsanguinated whole body without liver and gastrointestinal tract) of lambs.

3. Absolute synthesis rates (ASR) of tissue protein were 17, 19 and 42 g/d in the liver, skin and skeletal muscle respectively, and 112 g/d in the remainder. The ASR of whole-body protein, derived from the tissue values, was 146 g/d, i.e. 33 g/d per kg body-weight. The calculated whole-body protein FSR was 23·9%/d.

4. The relative percentage contribution of liver, skin and skeletal muscle to whole-body protein synthesis was 11·7, 13·1, and 29·0.

5. We concluded that tissue protein FSR in lambs were in exactly the same decreasing order, from visceral tissues to skeletal muscles, as observed in rats. The ovine FSR estimates and the partitioning of protein synthesis between tissues were in the same range as values recently obtained by flooding-dose experiments in immature rats, piglets, and even in chicks. These findings suggest that inter-species differences are rather limited.

The continuous balance between body protein synthesis and breakdown (protein turnover) regulates protein deposition, and hence commercial productivity for farm livestock. Over the past 15 years, studies of protein synthesis have been performed in pigs (Garlick *et al.* 1976; Edmunds *et al.* 1978; Simon *et al.* 1978; Reeds *et al.* 1980; Mulvaney *et al.* 1985; Sève *et al.* 1986*b*), sheep (Soltész *et al.* 1973; Arnal, 1977; Buttery *et al.* 1977; Davis *et al.* 1981; Bryant & Smith, 1982; Patureau-Mirand *et al.* 1985; Schaefer *et al.* 1986), cattle (Lobley *et al.* 1980), heifers (Hammond *et al.* 1987) and beef steers (Lobley *et al.* 1987). However, quantitative information for both whole-body and tissue protein synthesis is scarce since the constant-infusion technique used in most of these experiments has severe limitations, extensively detailed elsewhere (Waterlow *et al.* 1978). In addition, there is some controversy over the partitioning of protein synthesis between tissues in several species (Lobley *et al.* 1980). Consequently, the study of mechanisms controlling protein deposition in the whole body, the viscera and tissues of economical interest (muscles) is poorly documented in farm animals.

We recently reported protein fractional synthesis rate (FSR) in the gastrointestinal tract of young preruminant lambs by a large-dose injection of L-[3,4(*n*)-^{3}H]valine (Attaix & Arnal, 1987). The present study describes the rates of protein synthesis in liver, skin, skeletal muscle and whole body of these animals. The large-dose technique increases considerably the accuracy of protein FSR measurements in lambs (Attaix *et al.* 1986*b*; Attaix & Arnal, 1987) so that reliable absolute synthesis rates (ASR) can be determined. We attempted therefore to provide for the first time a meaningful estimation of the relative contribution of some major tissues to whole-body protein synthesis (WBPS) in a farm livestock species.

MATERIALS AND METHODS
Animals and measurements

The six Ile de France × Romanov–Limousin male lambs (4·5 (SE 0·2) kg live weight; 7–8 d of age) were those used in Expt 1 of our recent report on protein synthesis in the gastrointestinal tract of the young lamb (Attaix & Arnal, 1987). Animals were given only a commercial milk replacer *ad lib.* ('Agnodor'; Union Univor, Paris) supplied by nipple feeders. The technique for measuring protein synthesis has been described previously in detail (Attaix & Arnal, 1987). In brief, the lambs were given a single intravenous injection of 17·1 mmol unlabelled L-valine/5 kg body weight, i.e. approximately 14·4 times the whole body free valine content (Attaix *et al.* 1986 *b*). This large amount of valine was combined with L-[3,4(*n*)-^{3}H]valine (specific radioactivity 25–50 Ci/mmol; Amersham International plc, Amersham, Bucks) to give 0·45 mmol and 95 μCi/ml saline (9 g sodium chloride/l). Blood samples were then regularly collected and plasma isolated. Pairs of anaesthetized animals were killed 5, 13 and 30 min after injection. The thoracic and abdominal cavities were immediately opened. The liver was excised, immersed in ice cold saline and cut into small pieces to minimize blood contamination. Portions were then blotted and frozen in liquid nitrogen. The mainly red (M. diaphragma), the predominantly white (M. tensor fasciae latae (M. TFL)) fibre-type muscles and a 100 × 100 mm piece of shaved skin on the back of the animals were dissected and immediately frozen. The remainder of the lambs (exsanguinated whole body, without the gastrointestinal tract and liver) was chopped up and rapidly frozen. The frozen remainder was minced in liquid nitrogen with a meat grinder. A portion was taken for further analysis and stored with tissue samples at $-15°$.

The specific radioactivity of valine, either free in plasma or in tissue homogenates (SA), and covalently bound in protein (SB) was determined (Attaix *et al.* 1986 *b*; Attaix & Arnal, 1987). The protein FSR (%/d) was calculated from the equation:

$$100 \times SB(t) = FSR(SA \times t) + (100 \times SB t_0),$$

where t is the incorporation time in days, and SA is the specific activity of free valine between t_0 and t, determined according to Garlick *et al.* (1980). FSR were calculated assuming that the SA values in the plasma ($FSR_{Minimum(Min)}$ calculation) and in the tissue homogenate ($FSR_{Maximum(Max)}$ calculation) defined that of valyl tRNA.

The ASR (g protein synthesized/d) were calculated by multiplying tissue protein content, obtained by a Kjeldahl procedure (N × 6·25), by FSR_{Max}. For the remainder ASR, allowance was made for the skin and skeletal muscle samples that had been removed for measuring protein FSR.

Statistical procedures

The time-courses for SA and SB were analysed by linear regression. Protein FSR, i.e. the slopes of best-fit of SB(*t*) *v.* (SA × *t*) were compared according to Snedecor & Cochran (1971). The standard errors for slopes and intercepts of regression lines were computed as described by Sokal & Rohlf (1969).

RESULTS

The time-courses for SA and SB were linear after [^{3}H]valine injection (Fig. 1). A small decline (maximum 17%) was observed for SA in the plasma and all tissue homogenates, between 5 and 30 min. The SA ratio, tissue homogenate:plasma, was at a minimum in the skin (0·82(SE 0·02)), ranging from 0·85 (SE 0·02) to 0·88 (SE 0·01) in liver and muscles, and was 0·96 (SE 0·01) in the remainder of the lambs.
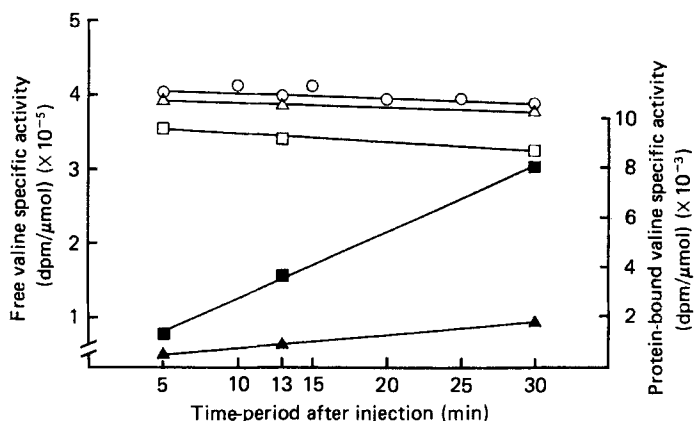
Fig. 1. Time-courses for the free (SA; $\bigcirc$, $\triangle$, $\square$) and the protein-bound (SB; $\blacktriangle$, $\blacksquare$) [³H]valine specific radioactivity (disintegrations/min (dpm) per μmol) in the plasma ($\bigcirc$), the remainder (exsanguinated whole body without liver and gastrointestinal tract) ($\triangle$, $\blacktriangle$) and liver ($\square$, $\blacksquare$) of lambs. Plasma values are means for two to six lambs. Tissue values are means for two animals. The equations describing the relation between SA or SB and time (t, min) were in liver:

$$SA = 360105(\text{SE } 12869)\,t - 1281(\text{SE } 674), \quad SB = 269(\text{SE } 20)\,t + 130\,(\text{SE } 385),$$

and in the remainder:

$$SA = 393585(\text{SE } 14821)\,t - 685(\text{SE } 776), \quad SB = 52(\text{SE } 2)\,t + 229(\text{SE } 48).$$

Table 1. *Protein-bound valine and tissue protein fractional synthesis rates (FSR) in preruminant lambs*

(Mean values with their standard errors for six lambs)

| | | FSR (%/d) | | | |
| | | Min* | | Max* | |
Tissue	Protein bound valine (mg/g)	Mean	SE	Mean	SE
M. diaphragma	56	18·4	1·8	21·6	1·7
M. tensor fasciae latae	56	20·1	2·3	22·9	2·6
Liver	61	97·8	7·8	115·0	7·4
Skin	49	20·1	2·1	24·1	2·7
Remainder †	49	18·9	1·1	19·6	0·9

Min, Max, minimum and maximum estimates of FSR obtained using the specific radioactivity of free valine in the plasma and the tissue homogenate respectively.
* FSR_{Min} and FSR_{Max} were not significantly different in any tissue ($P > 0·05$).
† Exsanguinated whole body without liver and gastrointestinal tract.

FSR_{Min} and FSR_{Max} were not significantly different ($P > 0·05$) in any tissue (Table 1). The highest values for FSR were observed in the liver (97·8–115·0 %/d), and the lowest in the remainder (18·9–19·6 %/d). No significant differences were observed between protein FSR in the white M. TFL and the red M. diaphragma ($P > 0·05$).

The whole-body protein ASR was calculated as the sum of the protein ASR in the gastrointestinal tract of lambs used in the present experiment (16·8 (SE 0·7) g/d, Attaix & Arnal, 1987), the liver and the remainder (Table 2). We estimated that our lambs synthesized 146·4 g protein/d, i.e. 32·7 (SE 0·5) g/d per kg body-weight, or 47·5 (SE

Table 2. *Tissue and whole-body protein content, protein absolute synthesis rates (ASR) and percentage contributions of tissues to whole-body protein mass (WBPM) or synthesis (WBPS) in preruminant lambs*

(Mean values with their standard errors for six lambs)

Tissue	Protein content (g)		ASR (g/d)*		WBPM (%)	WBPS (%)
	Mean	SE	Mean	SE		
Muscle†	191·0	9·1	42·5	2·0	31·2	29·0
Liver	14·9	1·3	17·1	1·5	2·4	11·7
Skin	79·2	3·4	19·1	0·8	13·0	13·1
Remainder‡	572·7	19·8	112·5	3·9	93·6	76·8
Other tissues§	302·4	—	50·9	—	49·4	34·7
Gastrointestinal tract‖	24·2	1·0	16·8	0·7	4·0	11·5
Whole body	611·8	21·6	146·4	5·7	100	100

* ASR = Tissue protein content $\times$ FSR$_{Max}$ (see Table 1).
† Assuming skeletal muscle represents 36 % of empty body-weight (Bénévent, 1971) and that protein fractional synthesis rate (FSR) is the mean value of M. diaphragma and M. tensor fasciae latae FSR.
‡ See Table 1.
§ Remainder − (skin + muscle).
‖ From Attaix & Arnal (1987).

0·6) g/d per kg body-weight$^{0.75}$. The mean whole-body protein FSR, derived from the WBPS divided by the body protein content, was 23·9 %/d.

The relative percentage contribution of liver, skin and muscle to WBPS was 11·7, 13·1 and 29·0 respectively (Table 2). When protein ASR were calculated from FSR$_{Min}$, these relative contributions were in a very similar range (10·6, 12·0 and 26·8 % for liver, skin and muscle respectively). With both calculations, the liver and skin together synthesized approximately as much protein as muscle.

DISCUSSION

Validity of measurements of whole-body protein ASR

Two main ways of measuring WBPS rates in large animal species have been previously reported. The most commonly used is the constant intravenous infusion of a labelled amino acid. Through blood samples, the measurement of the irreversible loss rate of the label provides a simple and rapid method from which the rate of WBPS can be calculated. The second way is to determine protein FSR and then ASR in some major tissues, after infusion or injection of tracer doses of labelled amino acids. Then, an approximate rate of WBPS is calculated by adding the ASR values. However, both procedures have severe limitations and uncertainties which have been discussed in detail elsewhere (Waterlow *et al.* 1978; Garlick, 1980; Lobley *et al.* 1980).

The present paper differs fundamentally from previous studies performed in farm animals in that (1) we used a large-dose technique and (2) we derived WBPS from direct FSR measurements in all tissues, including the viscera-free carcass. This has not been done previously in infused farm animals. However, in fairness the size of animals used in infusion experiments has usually been at least six times larger than those used in the present study and such experiments would therefore be accompanied by technical difficulties. The advantages of measuring protein FSR in lambs with a flooding-dose technique have been previously discussed (Attaix *et al.* 1986*b*; Attaix & Arnal 1987). Point (2) is of interest since

the validity of protein FSR measurement in the remainder of our lambs may be questionable. The time elapsed between slaughter and the final freezing of the remainder in liquid N_2 was 11–16 min. This was considerably longer than the time necessary for cooling individual tissue samples (2–7 min). Thus, SA or SB, or both, might be altered which would result in a misleading remainder protein FSR. We have previously reported no change or a small decrease for SB with time after slaughter (5–15 min) in jejunal tissue protein (Attaix *et al.* 1986*b*). SA measurements in the remainder (Fig. 1) suggested that little if any alteration for SA occurred by unlabelled valine arising from protein breakdown. Consequently, the SA ratios, remainder homogenate:plasma, remained very high (0·96 (SE 0·01)). We concluded therefore that the error made in the remainder protein FSR determination, and subsequently in WBPS, was in the same range as for other tissues.

Tissue protein FSR

From the present findings, and those previously reported for the gastrointestinal tract of our lambs (Attaix & Arnal, 1987), tissue protein FSR in preruminant lambs can be classified in the decreasing order: liver, small intestine, stomach (abomasum), large intestine, smooth muscle (abomasal musculosa), skin, whole body, skeletal muscles. This decreasing order of FSR corresponded exactly to that reported in large-dose experiments in tissues of 100 g (McNurlan & Garlick, 1980) and of 3- to 105-week-old rats (Goldspink & Kelly, 1984; Goldspink *et al.* 1984; Lewis *et al.* 1984), except for liver and small intestine. Rats used in those experiments were weaned animals. It has been reported in lambs (Combe *et al.* 1979), rats (Reeds *et al.* 1982) and piglets (Sève *et al.* 1986*b*) that weaning enhanced small intestinal protein FSR. Sève *et al.* (1986*b*) found that liver protein FSR was higher than the small intestinal value in immature piglets, as in our lambs; the reverse pattern was observed in sucking lean Zucker rats (Reeds *et al.* 1982) (Table 3). We have evidence that liver protein FSR fell abruptly with increasing age in sucking lambs whereas small intestinal FSR remained very high (Attaix *et al.* 1986*a*). The apparent discrepancy between liver and small intestinal protein FSR, within different immature species, could therefore be related to developmental changes.

Another apparent difference between lambs and rats is the skin protein FSR (Table 3). However, the 63·6 %/d value reported by Preedy *et al.* (1983) referred only to soluble protein in 0·3 M-sodium hydroxide. By contrast, our value (24·1 %/d) compares favourably with the estimates reported in infused lean Zucker rats (21·1 %/d) by Lobley *et al.* (1978) and newborn lambs (29·4 %/d) by Patureau-Mirand *et al.* (1985). In addition, Sève *et al.* (1986*a*) obtained a 25·5 %/d estimate in unweaned pigs, with a flooding-dose of [³H]phenylalanine.

All measurements reported in Table 3 were obtained with flooding-dose techniques in young mammals or chicks. An absolute comparison of these values has no sense since the stage of development of the animals varied according to the species. In addition, protein FSR depends closely on age (Waterlow *et al.* 1978). However, although tissue protein FSR was generally slightly higher in rats than in lambs, piglets or chicks, the range of protein FSR for a given tissue within the four animal species, including birds, suggested that inter-species differences were rather limited.

Contribution of tissues to WBPS

Since the reviews of Lobley *et al.* (1980) and Reeds & Lobley (1980) little advance has been made in comparisons of tissue contributions to WBPS between species, due to the lack of reliable reports available (Waterlow, 1984). This point is, however, of importance. If the contribution of an individual tissue varies between species, one can postulate that the control mechanisms of protein synthesis, or their amplitude, must also differ. Consequently,

Table 3. *A summary of some major tissue protein fractional synthesis rates (FSR, %/d) obtained with flooding-dose experiments in several animal species*

(Values in parentheses refer to percentage contributions of tissues to whole-body protein synthesis)

Species...	Lamb		Rat		Rat		Piglet		Chick	
Stage of growth...	7–8 d		18 d		100–130 g		10 d		23 d	
Tissue										
Muscle	22	(29)	22	(33)	17	(25)*	16	(35)¶	—	—
Liver	115	(12)	70	(6)	86	(15)*	85	(13)¶	73	(11)
Skin	24	(13)	—	—	64	(18)*	26	(14)¶	—	—
Small intestine	88†	(9)	100‡	(16)	103	(9)§	59‖	(13)¶	68†	(14)
Source**...	1, 2		3		4, 5		6		7	

* Soluble protein in 0·3 M-sodium hydroxide.
† Mean values of duodenum, jejunum and ileum FSR.
‡ Mixed small and large intestines.
§ Recalculated from McNurlan & Garlick (1980), assuming that the whole-body protein content was 160 g/kg body-weight (Millward *et al.* 1981).
‖ Duodenum.
¶ B. Sève, unpublished results.
** Source: 1, Present study. 2, Attaix & Arnal (1987). 3, Reeds *et al.* (1982). 4, McNurlan & Garlick (1980). 5, Preedy *et al.* (1983). 6, Sève *et al.* (1986*a*, *b*). 7, Muramatsu *et al.* (1987).

protein deposition as well as the response of species to manipulations of protein metabolism could also vary (Lobley *et al.* 1980).

Table 3 shows the partitioning of WBPS between tissues in lambs, rats, pigs (B. Sève, personal communication) and chicks. We compared only these estimates, obtained by flooding-dose procedures, to avoid inter-method variation. Furthermore, it is noticeable that tissue and WBPS in lambs, chicks and 18-d-old lean Zucker rats (Reeds *et al.* 1982), were derived from the same experiment, so that the summation to 100 % was realistic. Finally, in these studies a Kjeldahl method was used to estimate the whole-body protein mass. Indeed, the partitioning of total synthesis between tissues depends closely on the technique used for estimating body protein content and then calculating WBPS. This can be demonstrated as follows. The contribution of tissues with high protein FSR (liver, intestine) to WBPS has probably been overestimated in previous experiments performed in rats (McNurlan & Garlick, 1980; Goldspink & Kelly, 1984; Goldspink *et al.* 1984). In these experiments, the whole-body protein content was measured by the method of Lowry *et al.* (1951) and exhibited very low values; for example, only 9·3 % live weight was protein in the experiment of McNurlan & Garlick (1980). It is generally accepted that young male rats contain 16–17 % crude protein (Munro & Fleck, 1969). McNurlan & Garlick (1980) calculated WBPS in 100-g rats by multiplying the whole-body protein FSR (0·336/d) by its corresponding protein mass (9·3 g). Accordingly, they found that their animals would synthesize 3·13 g protein/d and that the relative contribution of liver (protein ASR = 743 mg/d) and small intestine (protein ASR = 477 mg/d) to WBPS would be 23·7 and 15·2 % respectively. Assuming that the rat body protein content is 16 %, WBPS is actually 5·38 g/d. Consequently, in this experiment, liver and small intestine contribute only 13·8 and 8·9 % of total synthesis respectively.

Skeletal muscle, skin, liver (except in immature rats) or small intestine (Table 3) provided a fairly constant proportion of WBPS (25–35, 13–18, 11–15 and 9–16 % respectively). The added contribution of visceral tissues, liver and the small intestine, was in the range

21–26 %, including birds. In addition, skin and the visceral tissues together synthesized a greater proportion of protein than muscle in sucking lambs or piglets. Young rats exhibited the same pattern (McNurlan & Garlick, 1980; Preedy *et al.* 1983), even when the limitations quoted above are taken into account. All these observations clearly support very small species differences, in immature and young animals.

REFERENCES

Arnal, M. (1977). *European Association for Animal Production* **22**, 35–37.

Attaix, D. & Arnal, M. (1987). *British Journal of Nutrition* **58**, 159–169.

Attaix, D., Manghebati, A. & Arnal, M. (1986a). *Reproduction, Nutrition, Développement* **26**, 703–704.

Attaix, D., Manghebati, A., Grizard, J. & Arnal, M. (1986b). *Biochimica et Biophysica Acta* **882**, 389–397.

Bénévent, M. (1971). *Annales de Biologie Animale, Biochimie, Biophysique* **11**, 5–39.

Bryant, D. T. W. & Smith, R. W. (1982). *Journal of Agricultural Science, Cambridge* **98**, 639–643.

Buttery, P. J., Beckerton, A. & Lubock, M. H. (1977). *European Association for Animal Production* **22**, 32–34.

Combe, E., Attaix, D. & Arnal, M. (1979). *Annales de Recherches Vétérinaires* **10**, 436–439.

Davis, S. R., Barry, T. N. & Hughson, G. A. (1981). *British Journal of Nutrition* **46**, 409–419.

Edmunds, B. K., Buttery, P. J. & Fisher, C. (1978). *Proceedings of the Nutrition Society* **37**, 32A.

Garlick, P. J. (1980). In *Comprehensive Biochemistry*, vol. 19B, part 1, pp. 77–152 [M. Florkin and E. H. Stotz, editors]. Amsterdam: Elsevier.

Garlick, P. J., Burk, T. L. & Swick, R. W. (1976). *American Journal of Physiology* **230**, 1108–1112.

Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980). *Biochemical Journal* **192**, 719–723.

Goldspink, D. F. & Kelly, F. J. (1984). *Biochemical Journal* **217**, 507–516.

Goldspink, D. F., Lewis, S. E. M. & Kelly, F. J. (1984). *Biochemical Journal* **217**, 527–534.

Hammond, A. C., Huntington, G. B., Reynolds, P. J., Tyrrell, H. F. & Eisemann, J. H. (1987). *Journal of Animal Science* **64**, 420–425.

Lewis, S. E. M., Kelly, F. J. & Goldspink, D. F. (1984). *Biochemical Journal* **217**, 517–526.

Lobley, G. E., Connell, A. & Buchan, V. (1987). *British Journal of Nutrition* **57**, 457–465.

Lobley, G. E., Milne, V., Lovie, J. M., Reeds, P. J. & Pennie, K. (1980). *British Journal of Nutrition* **43**, 491–502.

Lobley, G. E., Webster, A. J. F. & Reeds, P. J. (1978). *Proceedings of the Nutrition Society* **37**, 20A.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, P. J. (1951). *Journal of Biological Chemistry* **193**, 265–275.

McNurlan, M. A. & Garlick, P. J. (1980). *Biochemical Journal* **186**, 381–383.

Millward, D. J., Brown, J. G. & Odedra, B. (1981). In *Nitrogen Metabolism in Man*, pp. 475–494 [J. C. Waterlow and J. M. L. Stephen, editors]. London: Applied Sciences Publishers.

Mulvaney, D. R., Merkel, R. A. & Bergen, W. G. (1985). *Journal of Nutrition* **115**, 1057–1064.

Munro, H. N. & Fleck, A. (1969). In *Mammalian Protein Metabolism*, vol. 3, pp. 423–525 [H. N. Munro, editor]. New York: Academic Press.

Muramatsu, T., Tasaku, O., Furuse, M., Tasaki, I. & Okumura, J. I. (1987). *Biochemical Journal* **246**, 475–479.

Patureau-Mirand, P., Bernard, O., Prugnaud, J. & Arnal, M. (1985). *Reproduction, Nutrition, Développement* **25**, 1061–1073.

Preedy, V. R., McNurlan, M. A. & Garlick, P. J. (1983). *British Journal of Nutrition* **49**, 517–523.

Reeds, P. J., Cadenhead, A., Fuller, M. F., Lobley, G. E. & McDonald, J. D. (1980). *British Journal of Nutrition* **43**, 445–455.

Reeds, P. J., Haggarty, P., Wahle, K. W. J. & Fletcher, J. M. (1982). *Biochemical Journal* **204**, 393–398.

Reeds, P. J., & Lobley, G. E. (1980). *Proceedings of the Nutrition Society* **39**, 43–52.

Schaefer, A. L., Davis, S. R. & Hughson, G. A. (1986). *British Journal of Nutrition* **56**, 281–288.

Sève, B., Peignier, Y. & Lebreton, Y. (1986a). *Diabète et Métabolisme, Paris* **12**, Abstract 118.

Sève, B., Reeds, P. J., Fuller, M. F., Cadenhead, A. & Hay, S. M. (1986b). *Reproduction, Nutrition, Développement* **26**, 849–861.

Simon, O., Münchmeyer, R., Bergner, H., Zebrowska, T. & Buraczewska, L. (1978). *British Journal of Nutrition* **40**, 243–252.

Snedecor, G. W. & Cochran, W. G. (1971). *Méthodes Statistiques*, 6th ed. Paris: Association de Coordination Agricole.

Sokal, R. R. & Rohlf, F. J. (1969). *Biometry*. San Francisco: W. H. Freeman.
Soltész, G., Joyce, J. & Young, M. (1973). *Biology of the Neonate* **23**, 139–148.
Waterlow, J. C. (1984). *Quarterly Journal of Experimental Physiology* **69**, 409–438.
Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978). *Protein Turnover in Mammalian Tissues and in the Whole Body*. Amsterdam: Elsevier, North Holland.