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Development of a Sheep Hind-Limb Muscle Preparation for Metabolic Studies

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Abstract

A sheep hind-limb preparation used for the study of muscle metabolism by arteriovenous (AV) difference procedures was validated by identifying the muscles which contribute to venous drainage at different positions along the lateral saphenous vein. Dissection of the hind limbs of six mature sheep (three wethers and three ewes) showed that venous blood from the plantar group (*M. gastrocnemius*, *M. soleus*, *M. plantaris*, *M. flexo digitorum profundus*), and from *M. semitendinosus*, *M. biceps femoris*, *M. gracilis*, *M. pectineus* and *M. adductor* muscles entered the lateral saphenous vein but the position of the tip of the blood sampling catheter was found to be critical. In order to sample venous blood from all of the muscles listed above, and to minimize the contribution of blood from non-muscular tissues, blood samples must be taken 25–26 cm from the junction of the cranial and caudal branches of the lateral saphenous vein (for average size sheep of body length about 108 cm and height at withers about 73 cm).

The estimation of sheep hind-limb muscle mass is a necessary concomitant of AV difference studies, and a combined tritiated water and dye-dilution procedure has been used to measure both muscle mass and blood flow.

The muscle mass estimated *in vivo* by this technique was closely similar to the true muscle mass obtained by dissection, the range of values of the difference between true and calculated muscle mass expressed as percentage of the true mass being 0·5–16%. It is concluded that these techniques are sufficiently accurate for use in the quantitation of exchange of metabolites across the hind-limb muscle preparation.

Patterns of amino acid uptake and release by muscle need to be related to the amino acid profile of the tissue, and the amino acid content of a representative muscle, *M. biceps femoris*, was determined, and the results compared with published data.

Introduction

The *in vivo* technique described by Domanski *et al.* (1974) for the measurement of arteriovenous (AV) differences of circulating substrates across a sheep hind-limb muscle bed has been widely used (see Pethick *et al.* 1981). Major prerequisites of the technique are that the venous blood sampled should be fully representative of total venous drainage, and that the contribution of blood draining other tissues should be insignificant. Although both criteria would appear to be met by the procedure described by Domanski *et al.* (1974), data were not reported on which muscles contribute to the venous drainage, or on the importance of the position of the venous blood sampling catheter. Information on the vasculature of the hind limbs is surprisingly scanty (see Hecker 1974). There is evidence that blood flow through different groups of muscles in the hind limb is not identical (Oddy *et al.* 1981), and substrate uptake and release by these muscles may also differ. Further, it has been

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demonstrated that metabolic activity, such as protein synthetic rate, may vary between different muscle groups (Lobley *et al.* 1980; Bryant and Smith 1982).

Quantitative studies based on AV difference measurements require the simultaneous measurement of blood flow. The tritiated water (TOH) procedure of Pappenheimer and Setchell (1972) measures blood flow as volume of blood per unit time per unit weight of tissue. This means that if total blood flow per unit time is measured by a second, independent procedure, the mass of tissue can be estimated. The dye-dilution technique of Jorfeldt and Wahren (1971) measures total blood flow, and indeed has been used successfully to measure leg muscle blood flow in steers (Bell *et al.* 1974) and sheep (Jarrett *et al.* 1976). In both studies, however, blood flow to the whole hind limb was measured.

In the present study the feasibility of using both the TOH procedure of Pappenheimer and Setchell (1972), as modified by Oddy *et al.* (1981), and the dye-dilution technique for measuring blood flow to estimate muscle mass *in vivo* has been examined. The application of the dye technique to the sheep hind-limb preparation of Domanski *et al.* (1974) has not been described, and it was necessary to develop the technique for this preparation.

Evidence that amino acids may either be synthesized or catabolized in muscle has been derived by measuring amino acid uptake or output in relation to their relative occurrence in that tissue (Felig and Wahren 1971; Ruderman and Berger 1974). In these studies, and in most others on human muscle, the data of Kominz *et al.* (1954) on muscle amino acid composition was used. Coward and Buttery (1982) measured the amino acid content of sheep hemidiaphragm, but data on individual skeletal muscles of sheep are largely unavailable.

The four objectives of the present studies were to identify those muscles which contribute venous drainage to the lateral saphenous vein; to establish the best position of the catheter in the lateral saphenous vein for the collection of blood draining known muscles, with minimum contamination with blood from other tissues; to combine the TOH procedure for the measurement of blood flow with the dye-dilution technique for the estimation of muscle mass; to determine the amino acid composition of *M. biceps femoris*, a major muscle group in the sheep hind limb.

Materials and Methods

Experimental Animals

Mature crossbred (Border Leicester × Merino) ewes and wethers were housed indoors and fed lucerne chaff (8·2 MJ/kg dry matter; 13% crude protein) to calculated requirements (MAFF 1975). Feeding levels were adjusted, where necessary, to ensure that animals remained in good condition at roughly constant liveweight.

Preparation of Tissues

Three wethers and three ewes were used for this study. They were all of average frame, with height at withers ranging from 72 to 74 cm and body length of approximately 108 cm.

Each animal was heparinized (500 i.u./kg) and then killed with an overdose of sodium pentobarbitone. The animal was eviscerated, the external iliac vein and artery of each limb located and catheterized with polyethylene tubing (3·3 mm i.d. by 4·82 mm o.d.). The catheters were then clamped with haemostats and the hind limbs amputated at a level immediately anterior to the tuber coxae.

Arterial catheters were connected to reservoirs of warm saline which was allowed to flush through the connected vascular network under gravity. As the saline solution flowing out of the external iliac vein cleared of traces of blood, the arterial system was flushed with 10% (v/v) neutral formalin for fixing and 5% (w/v) borax to remove any blood clots in the vasculature.

Preparation of vascular casts

Siligard 170A and 170B silicone elastomer (Dow Corning Corporation, Michigan, U.S.A.), mixed in the ratio 4 : 5 respectively, were used as casting compounds. The raised proportion of the catalyst (170B) to base (170A) increased the curing time, thus allowing approximately 35 min before the mixture became too viscous to infuse. The mixture was degassed in a vacuum desiccator for 5 min at approximately 15 mmHg (2 kPa) prior to infusion. This prevented occlusion of small vessels with air bubbles during infusion. A 50-ml syringe was used to infuse the mixture into the external iliac artery. The casting compound was allowed to exude from the deep femoral vein for several minutes before both the femoral vein and the external iliac artery were clamped with haemostats. The limbs were left refrigerated for 24 h to allow the cast to set.

The grey-black colour of the elastomer aided visualization of the cast.

Dissection of hind limbs

Dissection was carried out on the hind limb to expose and trace the lateral saphenous vein (and its branches) and its continuation into the deep femoral and external iliac vein. Muscles with venous drainage connected to the lateral saphenous vein and its continuation were noted. Measurements between these connection points were taken.

Development of Dye-dilution Technique for Measurement of Blood Flow

The dye-dilution technique requires the continuous infusion of dye at constant rate to achieve a steady, or plateau concentration in blood (Andres *et al.* 1954). If there is significant recirculation of the dye, a plateau value will not be achieved. At equilibrium, the difference between the concentration of the diluted dye and recirculated dye remains constant.

The dye was infused into the distal section of the lateral saphenous vein. An incision was made in the skin over the lateral saphenous vein and the PVC sampling catheter (0·86 mm i.d. by 1·27 mm o.d., Dural Plastics, N.S.W.) inserted into the vein. The tip of the catheter was advanced up the vein until it was 26 cm from the junction of its cranial and caudal branches above the hock. The dye-infusion catheter (0·5 mm i.d. by 0·9 mm o.d.) was inserted into the vein through the same point of insertion as for the sampling catheter. Its tip was then advanced to 5 cm from the point of insertion. Patency could be maintained for up to 5 days.

The muscle-dissection studies showed that the weight of relevant muscles in the hind limb of a mature sheep would be approximately 1200 g. Further it was assumed that the magnitude of the upper range of the blood-flow rate through these muscles would be approximately 12 ml min^{-1} 100 g^{-1} (Oddy *et al.* 1981). From these values it was possible to calculate the concentration of dye in the infusate which, when diluted by blood flowing through the hind-limb muscles, would have a concentration value within the linear range of the standard curve. This concentration was 400 μg ml^{-1}. The infusion rate (ml min^{-1}) of the dye was then calculated to accommodate the probable range of dilution to be encountered and also to create turbulence in flow at the site of infusion. The calculated linear velocity of the dye infusate was approximately 17 cm sec^{-1}.

Attempts to introduce two sampling catheters at different levels in the deep femoral vein so that mixing of the infused dye with blood could be tested (Jorfeldt and Wahren 1971) were unsuccessful for several reasons. The first was that the vein is too small to accommodate two sampling catheters plus the infusion catheters and the second was that reduction of catheter size below 0·5 mm i.d. presented difficulties in finding suitable-sized wire guides for catheter introduction into the vein. Also, withdrawal of discrete samples over short time intervals was too slow to provide sufficient blood for spectrophotometric assay of the dye in plasma.

Indocyanine green was used as the dye. This is rapidly bound to blood proteins, particularly albumin. The concentration of dye in blood was measured spectrophotometrically. A standard curve was constructed by adding aliquots of a stock of dye to blood to give a concentration range of 0–10 μg ml^{-1}. Absorbance was read on plasma at 800 nm on a digital double-beam spectrophotometer (UV-150 Shimadzu, Kyoto, Japan). The concentration range of dye in blood found to obey Beer's Law was 0–6 μg ml^{-1}, and measurements were confined to this range of dye concentration.

Measurement of Blood Flow through Hind-limb Muscle

Two ewes and five wethers were prepared with indwelling catheters in the jugular vein, femoral artery and lateral saphenous vein, for the measurement of muscle blood flow by the TOH and dye-dilution procedures.

Tritiated water method

Blood flow was determined by the diffusion-equilibrium technique of Pappenheimer and Setchell (1972) as modified by Oddy *et al.* (1981).

Tritiated water, 10 ml (5250 kBq ml^{-1}), was mixed continuously in a vial using a magnetic stirrer. One ml (525 kBq) was infused in the first minute and the remainder infused at an exponentially decreasing concentration by adding to the vial for the remaining 59 min of continuous infusion a TOH diluent (52·5 kBq ml^{-1}) at a rate of 1 ml min^{-1}. The procedure produced an 'approximately square wave' of TOH concentration until equilibrium between blood and muscle water was achieved.

During the infusion period, arterial and venous blood samples were withdrawn continuously from respective catheters (femoral artery and deep femoral vein) by a peristaltic pump at a rate of 1 ml min^{-1}. To avoid heparinizing the animal, heparin (500 i.u. ml^{-1}) was infused into the sampled blood (0·04 ml min^{-1}) at a point near the exit of the catheter. Blood was collected over 10-min intervals in tubes kept cold in an ice bath.

Blood samples (0·5 ml) were deproteinized with 1·5 ml perchloric acid (0·5 M). After centrifugation at 3000 *g* for 15 min in a capped centrifuge tube, 1 ml of the clear protein-free supernatant was added to 10 ml scintillation cocktail and counted for tritium in a liquid scintillation counter (Philips PW 4540).

Blood flow throughout the hind-limb muscles was calculated using the Fick equation of Kety and Schmidt (1945):

$$M = [C_v(\text{eq.}) \,.\, S] \,/\, [\textstyle\int_0^{t(\text{eq.})}(C_a - C_v)\mathrm{d}t] \times 100\,,$$

where M is muscle blood flow (ml min^{-1} 100 g^{-1}); C_a and C_v are the respective concentration of TOH in arterial and venous whole blood during approach to equilibrium; C_v(eq.) is the concentration of TOH per millilitre venous blood at equilibrium; S is the partition coefficient which is the ratio of tissue TOH concentration per gram to venous TOH concentration per gram at equilibrium. Tritiated water concentrations in tissue and in arterial and venous blood water are assumed to be equal at equilibrium, although the fractions of water in muscle tissue and blood differ.

Use of the values of Oddy *et al.* (1981) for the water content of muscle (74·2%) and femoral venous blood (83·4%), and a correction factor of 1·04 to account for dilution of blood with heparin, gave a value for S of 0·855.

Dye-dilution Technique

Immediately after blood flow measurement using TOH, the dye infusion line pumping sterile physiological saline at approximately 1·8 ml min^{-1} (range 1·63–1·99 ml min^{-1}) was connected to the dye-infusion catheter. A pre-infusion sample of approximately 17 ml blood was taken for blank and standard preparation. The standard was prepared by diluting 0·14 ml of the dye infusate to 10 ml with blood to give a concentration of 5·6 μg ml^{-1}.

The infusate was continuously mixed using a magnetic stirrer. The end of the infusion line in the sterile saline was then transferred to the dye infusate and the time for the dye to reach the point of insertion of the catheter was noted. Blood samples (6 ml) were withdrawn simultaneously from the arterial and leg venous catheters at 4, 6, 8 and 10 min after start of infusion. From preliminary studies, it was noted that equilibrium was reached 4 min after the start of the infusion (see Fig. 3). It was therefore possible to take blood samples at either 4 and 6 min or 5 and 7 min for blood flow calculations. The two extra pairs of samples were taken to check the constancy of concentration difference between diluted and recirculated dye, which reflects the effectiveness of the mixing of the dye in blood.

All blood samples including blanks and standards were centrifuged to separate plasma, and absorbance was determined spectrophotometrically at a wavelength of 800 nm. Samples which had dye concentrations in excess of that of the standard were diluted with corresponding plasma blanks and their absorbance redetermined.

Blood flow was calculated using the following equation:

$$T = [I(D - V)] \,/\, (V - A)\,,$$

where

T = the total muscle blood flow;
I = the infusion rate of the dye in ml min^{-1};
D = the concentration of the infusate (g ml^{-1});
V = the mean venous concentration of the dye at equilibrium;
A = the mean arterial concentration of the dye at equilibrium.

Measurement of Muscle Mass

Sheep were weighed and slaughtered under general anaesthesia with sodium pentobarbitone by severing the blood vessels in the neck region to allow bleeding. Each hind limb was weighed and each relevant muscle group dissected out, sealed in a plastic bag and weighed.

Amino Acid Composition of M. biceps femoris

Entire biceps femoris muscles were obtained from 11 exsanguinated mature Merino ewes, minced separately and representative portions were either freeze-dried, or oven-dried at 80°C for 24 h for dry matter determination.

Approximately 0·5 g of each freeze-dried sample was weighed and wrapped in a dried Whatman filter paper, placed in an extraction thimble and fat extracted by refluxing with ether in a Soxhlet unit for 24 h. The sample plus filter paper was hydrolysed under nitrogen by refluxing with 100 ml 6 M HCl at 100°C for 21 h. The hydrolysate was transferred to a 250-ml volumetric flask and made up to volume with acid-distilled water. An aliquot (4 ml) of the hydrolysate plus 2 ml of 1 mM norleucine standard was rotary evaporated under reduced pressure to remove HCl. The residue was dissolved in 20 ml of Dionex diluent (Dionex, California, U.S.A.). A subsample was then filtered through a 0·22 μm Millipore filter into a 2-ml Wheaton vial, capped and sealed with a crimper. A Dionex D-300 Amino Acid Analyser (Dionex) was used for analysis. The sample was eluted through a microbore column (Na^+ form) using the Dionex sodium citrate buffered eluents (Dionex). The amino acid data were corrected for incomplete recoveries using values determined for lysozyme, a protein of known amino acid content (Canfield 1963).

Results

Anatomy and Vasculature of Hind-limb Preparation

The lateral saphenous vein enters the space between the *M. biceps femoris* and the *M. semitendinosus* (Fig. 1*a*), and passes across the caudolateral surface of the popliteal lymph node before entering the deep femoral vein on the medial surface of the *M. biceps femoris*. The deep femoral vein continues through the *M. semimembranosus* into the external iliac (Fig. 1*b*).

The muscles that drain into the lateral saphenous vein and its continuation into the deep femoral vein include the plantar group (*M. gastrocnemius*, *M. soleus*, *M. plantaris*, *M. flexo digitorum profundus*), and also *M. semitendinosus*, *M. biceps femoris*, *M. semimembranosus*, *M. gracilis*, *M. pectineus* and *M. adductor*. Relevant distances between venous branches along the lateral saphenous and deep femoral vein are shown in Fig. 1*a*.

The distances between the junction of the cranial and caudal branches of the lateral saphenous vein and the point of entry into the latter of the major venous drainage from the *M. biceps femoris* were closely similar between animals, i.e. 20 ± 0·2 cm. The junction of the cranial and caudal branches of the lateral saphenous vein is an important reference point. Evidence was obtained from examination of vascular casts that the ratio of blood draining muscle to blood draining non-muscular tissues is significantly reduced if the sampling tip of the venous catheter is less than 20 cm from the reference point. Between 16 and 20 cm from the reference point the muscle venous blood sampled is largely the plantar group (*M. gastrocnemius*, *M. soleus*, *M. plantaris*, *M. flexo digitorum profundus*), and the lower portion of the *M. semitendinosus*. A catheter placed less than 16 cm from the reference point samples blood draining largely non muscular tissues. At the 20 cm mark, blood sampled includes drainage from the plantar group (*M. gastrocnemius*, *M. soleus*, *M. plantaris*, *M. flexo digitorum profundus*), and from *M. semitendinosus* and *M. biceps femoris*. Approximately 1 cm beyond the 20 cm mark the drainage may include that from the *M. semimembranosus*. To include the *M. semimembranosus*,

M. gracilis, *M. pectineus* and *M. adductor*, the tip of the sampling catheter must be located at least 25 cm from the reference point. At approximately 29 cm, the catheter tip reaches the external iliac vein, and the sampled blood includes that flowing via the femoral and medial saphenous vein together with blood from the coccygeal region.

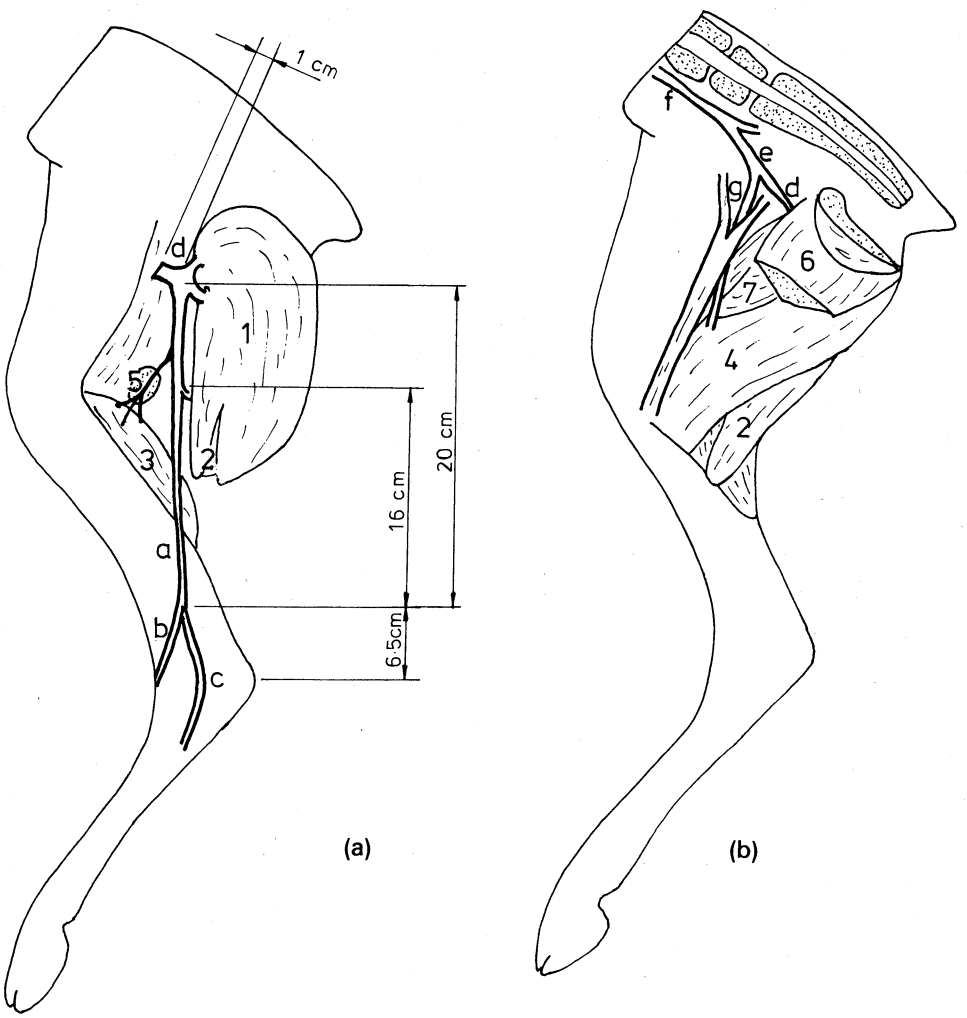

Fig. 1. Hind limb of mature sheep. (*a*) Lateral view: distances between venous branches along the lateral saphenous vein and deep femoral vein. *a*, Lateral saphenous vein; *b*, cranial branch; *c*, caudal branch; *d*, deep femoral vein; *1*, *M. biceps femoris* (medial); *2*, *M. semitendinosus* (lateral); *3*, *M. gastrocnemius* group 1; *5*, popliteal lymph node. (*b*) Medial view: extension of the deep femoral vein through the *M. semimembranosus* (*4*) from the lateral side and emerging on the medial side. Tip of the sampling catheter should be located at *d*. *e*, External iliac vein; *f*, caudal vena cava; *g*, femoral vein; *6*, *M. gracilis*; *7*, *M. adductor*.

In order to isolate a region which is predominantly muscle the sampling catheter tip must be fixed in position 26 cm from the junction of the cranial and caudal branch of the lateral saphenous vein. The recommended distance, 26 cm, is for average-sized mature sheep (body length approximately 108 cm and height at wither

approximately 73 cm). With smaller or larger animals, these distances would have to be reassessed.

Triated Water and Dye-dilution Curve

In most cases equilibrium concentration of TOH between blood and muscle water was achieved in the last 10 min of sampling time or a little after 60 min of infusion. A typical time course curve for TOH activity is shown in Fig. 2.

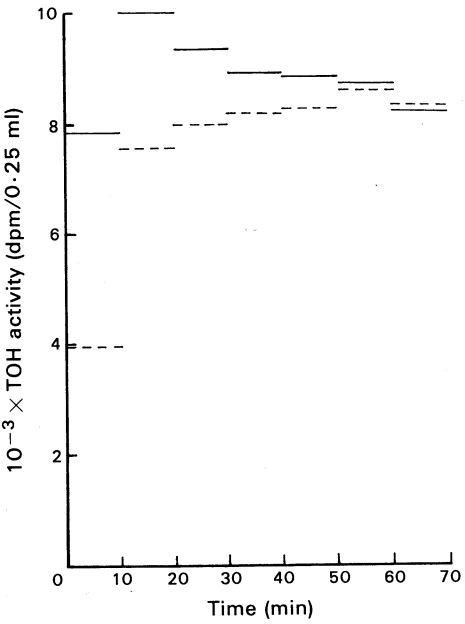

Fig. 2. Time course of TOH activity in arterial (——) and deep femoral venous blood (- - - - -).

The concentration of indocyanine green in arterial and deep femoral venous blood, plotted as a function of time, is shown for a typical animal in Fig. 3. It was found that equilibrium was reached by the fourth minute of dye infusion. The mean blood concentration of recirculated dye during this period was approximately 9% of the

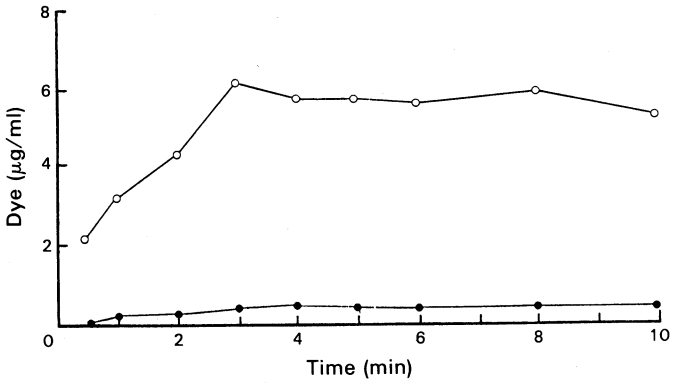

Fig. 3. Time course of indocyanine green concentration in arterial (●) and deep femoral venous blood (○).

mean blood concentration of dye sampled from the deep femoral vein. The coefficient of variation (within experiment) of the diluted dye at equilibrium was 3%, and of the recirculated dye 4%.

Sheep Liveweight, Leg Weight and Individual Muscle Weight

Sheep liveweight, leg weights and weights of relevant individual muscle group are shown in Table 1.

Table 1. Liveweights, leg weights, and individual muscle weights of seven sheep

Sheep No.:	1	2	3	4	5	6	7	Mean ± s.e.m.
Liveweight (kg)	43·6	32·3	49·2	33·2	49·2	52·6	65·7	46·5 ± 4·4
Leg weight (kg)	4·3	3·2	4·7	3·3	4·3	4·9	6·0	4·4 ± 0·4
Muscle weight (g)[A]								
M. biceps femoris	330	266	359	264	361	382	437	343 ± 24
M. semitendinosus	125	94	149	101	136	171	209	141 ± 15
M. semimembranosus	342	235	330	232	336	404	462	334 ± 31
M. gracilis	68	56	71	50	67	81	104	71 ± 7
M. adductor	136	128	163	124	151	179	182	152 ± 9
M. pectineus	31	42	34	37	44	56	62	44 ± 4
M. gastrocnemius	218	127	154	132	156	213	188	170 ± 14
M. soleus, *M. plantaris*, *M. flexo digitorum profundus*	218	174	169	170	161	233	217	192 ± 11

[A] Muscle drained by the lateral saphenous and deep femoral vein.

Muscle Blood Flow, Calculated and True Muscle Mass

Blood flows estimated by TOH and dye-dilution technique and muscle mass measured by dissection are summarized in Table 2. Using the paired *t*-test, there was no significant difference between the true and calculated muscle mass. The difference between true and calculated muscle mass, expressed as percentage of the true mass, yielded a mean ± s.e.m. of 7·5 ± 2·2%, range 0·5–16%.

Table 2. Blood flows measured by dye dilution (DD) and tritiated water (TOH) techniques, and calculated and measured weights of muscles drained by the lateral saphenous and deep femoral vein

Sheep No.	Blood flow DD (ml min^{-1})	Blood flow TOH (ml min^{-1} kg^{-1})	Muscle weight (g) Calculated	Muscle weight (g) Measured	Difference (as % of measured weight)
1	132·7	8·6	1543	1468	5·1
2	154·2	12·0	1285	1122	14·5
3	149·8	12·4	1208	1429	15·5
4	163·9	16·5	993	1110	10·5
5	104·0	7·5	1387	1412	1·8
6	114·0	6·6	1727	1719	0·5
7	125·0	6·4	1953	1861	4·9

Amino Acid Composition of M. biceps femoris

Mean values for each amino acid in *M. biceps femoris* muscle are shown in Table 3. The mean value (±s.e.m.) of muscle dry matter was 24·3 ± 0·44 g/100 g.

Table 3. Amino acid concentrations in sheep biceps femoris muscle expressed on a wet weight basis (mean ± s.e.m.) and as a proportion of total amino acids

Amino acid	Concn (mmol kg^{-1})	Concn as proportion of total amino acids (mol 100 mol^{-1})	Amino acid	Concn (mmol kg^{-1})	Concn as proportion of total amino acids (mol 100 mol^{-1})
Aspartate	148·8±0·28	10·4	Isoleucine	65·6±0·15	4·6
Threonine	84·7±0·05	5·9	Leucine	127·6±0·11	8·9
Serine	78·7±0·05	5·5	Tyrosine	36·6±0·03	2·6
Glutamate	225·6±0·16	15·7	Phenylalanine	51·3±0·06	3·6
Glycine	121·2±0·08	8·5	Lysine	121·2±0·18	8·5
Alanine	133·2±0·08	9·3	Histidine	41·2±0·06	2·9
Valine	83·2±0·18	5·8	Arginine	73·6±0·05	5·1
Methionine	38·5±0·02	2·7			

Discussion

The proposed modifications to the procedure of Domanski *et al.* (1974) for the measurement of AV differences across hind-limb muscle ensure the minimum of interference by venous drainage from other tissues. The finding that the tip of the venous sampling catheter must be precisely positioned is of practical importance. It is often possible to restore patency in blocked venous catheters by moving them, but this practice must be carried out cautiously with the hind-limb preparation: the allowable margin is about 0·5 cm in each direction.

The agreement between calculated and true muscle mass was better than expected, since the blood flow methods were used sequentially and even very small leg movements may cause appreciable changes in muscle blood flow. Part of the blood flow value included flow through skin and bone but the contribution from these two tissues is small (Domanski *et al.* 1974) and unlikely to exceed 5% (Oddy *et al.* 1981).

Although problems stemming from inadequate mixing of the dye and blood may be encountered using the dye-dilution technique as applied to this preparation (Ushioda *et al.* 1982), the sampling procedure used in this study enabled incorrect values to be excluded. Jorfeldt and Wahren (1971) found complete mixing of indocyanine green with venous blood when the dye was infused 22 cm in the distal direction from the inguinal ligament in the femoral vein of man. They measured dye concentrations at two different levels in the proximal femoral vein as a check.

The mean total blood flow of 135 ± 8 ml min measured by dye dilution in this study is similar to the value of 127 ± 17–152 ± 14 ml min reported by Bird *et al.* (1981). The similarity between present values for blood flow and those of Bird *et al.* (1981) is fortuitous, however, in that the latter values were for total hind-limb flow in smaller (35–40 kg) sheep. The present values, and those of Bird *et al.* (1981) are lower than the value of 195 ± 15 ml min^{-1} reported by Jarrett *et al.* (1976), but the latter workers did not give the body weights of their sheep. In both earlier studies, indocyanine green was infused into the external iliac artery and sampled from the corresponding vein.

The TOH technique yielded a mean muscle blood flow value of 10 ± 1 ml min^{-1}

100^{-1} g, almost identical to the mean flow of $9{\cdot}9 \pm 0{\cdot}9$ ml min^{-1} 100 g^{-1} reported by Oddy *et al.* (1981).

It is evident that both these techniques for measuring blood flow are satisfactory for use in the quantitation of exchange of metabolites across the hind-limb muscle preparation. It also appears that the two techniques are of sufficient accuracy to be used together to estimate the mass of the muscle being studied.

It is of interest to compare values obtained in this study for the amino acid composition of *M. biceps femoris* muscle with those of Kominz *et al.* (1954) where the amino acid values were expressed as percentage muscle protein sum of actin and myosin (see Ruderman and Berger 1974). Coward and Buttery (1982) have expressed their hemidiaphragm muscle protein values as ratios of individual amino acids to tyrosine. These ratios are used in Table 4 as bases for comparison between studies.

Table 4. Comparison of ratios of individual amino acids to tyrosine for sheep biceps femoris muscle obtained in present study with similar unpublished data of D. B. Lindsay (personal communication) and with data on the amino acid content of hemidiaphragm (Coward and Buttery, 1982) and actin plus myosin (Kominz *et al.* 1954)

Amino acid	Present study	D. B. Lindsay	Coward and Buttery	Kominz *et al.*
Aspartate	4·1	3·3	3·6	2·7
Threonine	2·3	1·8	2·2	2·1
Serine	2·2	1·8	1·9	–
Glutamate	6·2	4·8	–	6·2
Glycine	3·3	2·9	2·7	2·1
Alanine	3·6	3·2	3·2	3·4
Valine	2·3	2·1	2·8	1·8
Methionine	1·1	0·7	–	1·1
Isoleucine	1·8	1·5	1·4	1·1
Leucine	3·5	2·8	3·2	3·3
Proline	–	–	1·8	1·3
Phenylalanine	1·4	1·2	1·3	1·2
Lysine	3·3	2·9	2·7	6·6
Histidine	1·2	0·5	1·2	1·6
Arginine	2·0	1·9	1·7	7·1

The values are similar for most amino acids across the different studies, even though the methods of analysis were different. The outstanding discrepancies are in the ratios of glutamate, lysine and arginine to tyrosine. The values calculated from the data of Kominz *et al.* (1954) for the lysine : tyrosine and arginine : tyrosine ratios are 124 and 284% higher, respectively, than the mean of the corresponding values in the present study and those of D. B. Lindsay (personal communication) and Coward and Buttery (1982). A lower glutamate : tyrosine ratio has been observed by D. B. Lindsay (personal communication) but the value found in the present study is identical to that of Kominz *et al.* (1954).

The relative agreement between lysine values in the present study and those of D. B. Lindsay (personal communication) and Coward and Buttery (1982) suggest that the lysine value derived from the data of Kominz *et al.* (1954) is inaccurate. This conclusion impinges on the interpretation of data on amino acid exchange in

muscle in rats (Ruderman and Berger 1974) and man (Felig and Wahren 1971) where lysine has been used as a 'marker' amino acid based on the value for muscle lysine reported by Kominz *et al.* (1954).

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