

Magnitude of ouabain-sensitive respiration in the liver of growing, lactating and starved sheep

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1. Oxygen consumption and ouabain-sensitive respiration was measured for liver biopsies from lactating and non-lactating ewes and for hepatocytes isolated from mature, dry ewes. O_2 consumption, ouabain-sensitive respiration and $^{86}Rb^+$ uptake were also measured for hepatocytes isolated from lambs, fed adult sheep and adult sheep starved for 5 d.
2. Ouabain-sensitive respiration in the liver of ewes at peak lactation accounted for 45% of the total liver O_2 consumption. This percentage was 24–37% higher ($P < 0.05$) than measurements made during late lactation and during the non-lactating period.
3. Total O_2 consumption and ouabain-sensitive respiration rates of lamb hepatocytes were greater ($P < 0.05$) than similar measurements for hepatocytes isolated from adult sheep.
4. Ouabain-sensitive $^{86}Rb^+$ uptake by hepatocytes from fed sheep was up to six times greater ($P < 0.05$) than that by cells from starved sheep.
5. The magnitude of ouabain-sensitive respiration of hepatocytes from starved sheep was 62% lower ($P < 0.05$) than that for hepatocytes from fed sheep.

Background or maintenance energy expenditures of animals for support of protein turnover (Waterlow *et al.* 1978) and maintenance of sodium ion and potassium ion gradients across the plasma membrane (Glynn & Karlsh, 1975) have been considered to be simple, predictable, first-priority functions of body-weight (Milligan, 1971). However, quantitative knowledge of the metabolic components of maintenance-energy requirements and understanding of the constancy of these components is fragmentary.

The Na^+ pump (Na^+ , K^+ -ATPase; EC 3.6.1.3) participates in maintenance of ionic homeostasis within cells, catalysing conversion of 1 ATP to 1 ADP for every 3 Na^+ extruded from the cell and 2 K^+ pumped into the cell (Mandel & Balaban, 1981). This process may account for 30–70% of the total energy expenditure of animal tissues, particularly for tissues such as liver, gut epithelium, kidney and skeletal muscle (Lieberman *et al.* 1979; Balaban *et al.* 1980; Gregg & Milligan, 1982*a, b*; Van Dyke *et al.* 1983). Milligan (1971) suggested that tissue energy expenditure on ion transport may not be constant in animals under different physiological states. The purpose of the present experiment, therefore, was to measure the *in vitro* energy cost of Na^+ , K^+ -transport in hepatic tissue from growing and mature sheep. Second, the present study was conducted to determine if the maintenance cost of Na^+ , K^+ -transport in the liver changes in relation to the physiological demands imposed by lactation or starvation.

EXPERIMENTAL

Expt 1

Animals. Five Suffolk ewes, 2–3 years of age (62.4 (SE 1.9) kg), bearing twin lambs, were housed individually with their lambs in claiming pens (3.2 m²) through 8 weeks of lactation and for 2 weeks following lactation. The ewes were fed twice daily a total of 1.24 (SE 0.04)

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kg/d of both rolled barley (930 g dry matter (DM), 114 g crude protein (CP; nitrogen \times 6.25), 19.0 MJ gross energy (GE)/kg) and chopped bromegrass (*Bromus* spp.) hay (950 g DM, 111 g CP, 1603 MJ GE/kg). This level of feeding was maintained throughout lactation and during the dry period. Water and trace-mineralized salt were offered *ad lib*.

Milk yield was assessed at 4 weeks and 8 weeks of lactation using an oxytocin-hand-milking procedure. Between 08.00 and 09.00 hours on the day of collection, an intramuscular injection of 5 USP oxytocin was given and milk was stripped from both udder halves and discarded. The ewes were returned to their pens, their lambs were confined in wire cages to prevent sucking, and 2 h later the milking procedure was repeated and the milk weighed.

Liver-biopsy procedure. The liver-biopsy method used was similar to that described by Pearson & Craig (1980) for cattle and goats. The sheep were suspended and immobilized in a sling such that the abdominal contents forced the dorsal lobe of the liver against the rib cage. The puncture site, located at the 10th intercostal space 120 mm ventral to the backbone, was clipped and prepared for aseptic insertion of the Tru-Cut biopsy needle (Travenol Laboratories, St Louis, MO, USA). The biopsy area was infiltrated with lidocaine (20 ml/l), a small stab wound was made through the skin and the biopsy needle was directed caudo-ventrally through the abdominal wall into the dorsal lobe of the liver to remove the liver sample. The technique and location of the liver puncture was verified by laparotomy in other ewes before the commencement of the experiment. All biopsies were taken between 2 and 3 h after the morning feed. The liver biopsies were approximately 5–7 mm long, 2 mm wide and less than 1 mm thick. On removal of the liver samples they were washed in ice-cold Krebs–Henseleit buffer (pH 7.4 ± 0.01 , 37°), sliced freehand to less than 0.5 mm thickness with a microtome blade, then incubated in Krebs–Henseleit buffer (Dawson *et al.* 1969, pH 7.4 ± 0.01 , 37°) containing fatty acid-poor bovine serum albumin (20 g/l), 20 mM-Hepes and 10 mM-D-glucose for 5–10 min before being transferred to the oxygen electrode chamber. Tissue dry weight was determined at 90° .

Hepatocyte isolation and viability. Hepatocytes were isolated from one of the twin lambs from each ewe at 4 weeks of age (11.5 (SE 1.5) kg) and from the remaining lambs at 8 weeks of age (20.3 (SE 1.5) kg). They were removed from their dams immediately before surgery and were, therefore, not fasted at the time of liver perfusion.

Anaesthesia was induced and maintained with Halothane. The abdomen was opened by a lateral incision to the right flank distal to the ribs. The portal vein was ligated and a polyethylene catheter (inside diameter 0.86 mm, outside diameter 1.27 mm) was inserted 10 mm into the vein cranial to the ligature. The perfusion procedure used to isolate lamb hepatocytes was described previously (McBride & Milligan, 1985).

Hepatocytes were also isolated from two 3-year-old, non-pregnant, dry ewes (41.0 (SE 0.7) kg) which were given maintenance levels (950 (SE 36) g/d) of chopped bromegrass hay up to the time of surgery. In these animals, the caudate lobe of the liver was excised under Halothane anaesthesia, using the method of Clark *et al.* (1976). Following excision, the caudate lobe was perfused through a major vessel catheterized with a polyethylene catheter (inside diameter 0.86 mm, outside diameter 1.27 mm). Identical perfusion procedures and cell isolation techniques were used as described for the preparation of lamb hepatocytes (McBride & Milligan, 1985).

Detailed morphological and biochemical assessments of sheep hepatocytes isolated using perfusion techniques were reported by McBride & Milligan (1985). Results from this previous work indicated that hepatocyte viability could be adequately assessed by measuring trypan blue exclusion from the cells. The number of trypan-blue-stained cells were counted in an improved Neubauer counting chamber and expressed as a percentage of the total cell number (Seglen, 1976).

O₂ consumption and ouabain-sensitive and ouabain-insensitive respiration measurements. O₂

uptakes of the liver biopsies and hepatocytes were measured polarographically in a Yellow Springs Instrument model 53 O₂ electrode assembly. After 5–10 min of pre-incubation in air-saturated Krebs–Henseleit buffer (pH 7.40 ± 0.01, 37°), a liver biopsy or a 100 μl portion of the hepatocyte cell suspension was introduced into the electrode containing 4 ml air-saturated Krebs–Henseleit buffer (pH 7.40 ± 0.01, 37°, 700 mmHg, 180 nmol O₂/ml; Umbreit *et al.* 1964) containing fatty acid-poor bovine serum albumin (20 g/l), 20 mM-Hepes and 10 mM-D-glucose. Initial O₂ consumption was measured for 15 min, then ouabain was injected into the chamber to give a final concentration of 10⁻⁸ to 10⁻³ M. The O₂ consumption of the ouabain-treated samples was measured for a further 20–30 min. The difference between initial and ouabain-insensitive respiration was taken to represent ouabain-sensitive respiration. Percentage inhibition of respiration by ouabain was calculated using the ratio, ouabain-sensitive respiration: initial O₂ consumption rate. A dose-response curve for ouabain was constructed and all subsequent measurements of ouabain-sensitive respiration were made at concentrations of 1 × 10⁻⁴ M-ouabain.

Measurements of ouabain-sensitive ⁸⁶Rb⁺ uptake. The rates of ⁸⁶Rb⁺ uptake by hepatocytes from an 8-week-old lamb were measured in 1.9 ml of a gassed (O₂-carbon dioxide; 95:5, v/v) Krebs–Henseleit incubation buffer (pH 7.40 ± 0.01, 37°) containing fatty acid-poor bovine serum albumin (20 g/l), 20 mM-Hepes, 10 mM-D-glucose, 2.5 μCi/ml ⁸⁶Rb⁺ (New England Nuclear, Boston, MA) and 0.1 mM-RbCl. Duplicate 100 μl portions of the hepatocyte preparation were added to incubation buffers containing 10⁻⁸ to 10⁻³ or 0 M-ouabain, and incubation was continued for 10 min in a shaking water-bath (37°). The difference between undisturbed ⁸⁶Rb⁺ uptake and ⁸⁶Rb⁺ uptake of the hepatocytes subjected to ouabain inhibition was termed ouabain-sensitive ⁸⁶Rb⁺ uptake. Percentage inhibition of ⁸⁶Rb⁺ uptake by ouabain was calculated using the ratio, ouabain-sensitive ⁸⁶Rb⁺ uptake: total ⁸⁶Rb⁺ uptake. Dose-response curves were constructed by plotting percentage of maximum inhibition of ⁸⁶Rb⁺ uptake by ouabain *v.* ouabain concentration. Additionally, the time-course of inhibition of ⁸⁶Rb⁺ uptake by ouabain was assessed in 10⁻⁴ M-ouabain at 1, 5, 15, 30 and 60 min of incubation in a shaking water-bath.

In all determinations, ⁸⁶Rb⁺ uptake was stopped by filtration of the cells on to polycarbonate filters (8 μm pore size, Nucleopore, Minneapolis, MI, USA). The cells and filters were washed three times with ice-cold phosphate-buffered saline (pH 7.40 ± 0.01, 10 mM-NaH₂PO₄·H₂O, 8.5 g NaCl, 0.1 mM-RbCl), transferred to 15-ml plastic scintillation vials and digested in 1 ml Protosol (New England Nuclear) at 55° for 20 min. Glacial acetic acid (50 μl) was added to decolour the samples, then 10 ml Unisolve 1 scintillation fluid (Terochem Laboratories Ltd, Edmonton) were added to the vials. The samples were immediately counted for radioactivity on a Nuclear Chicago Mark 1 scintillation counter using balance-point counting with a 20:1 dynamic range window.

Expt 2

Animals. Four Suffolk wethers, all weighing 50 kg and of 1 year of age, were fed daily 1.0 kg chopped bromegrass hay (950 g DM, 140 g CP and 16.6 MJ GE/kg) in two equal portions twice (08.00 and 16.00 hours) daily to achieve maintenance. Another group of 1-year-old Suffolk wethers (49 kg) were starved for 5 d. Both groups of animals had free access to water and trace-mineralized salt.

Whole-animal O₂ consumption rates were measured for each animal over a 10 h period (Young *et al.* 1975). These measurements were made during the 5th day of starvation for the starved animals. Simultaneous measurements of whole-animal O₂ consumption were made with the fed animals. The sheep were subjected to surgery on the morning of the sixth day of starvation or 3 h after the morning feeding.

Hepatocyte isolation and measurements. The caudate-lobe-perfusion procedures described

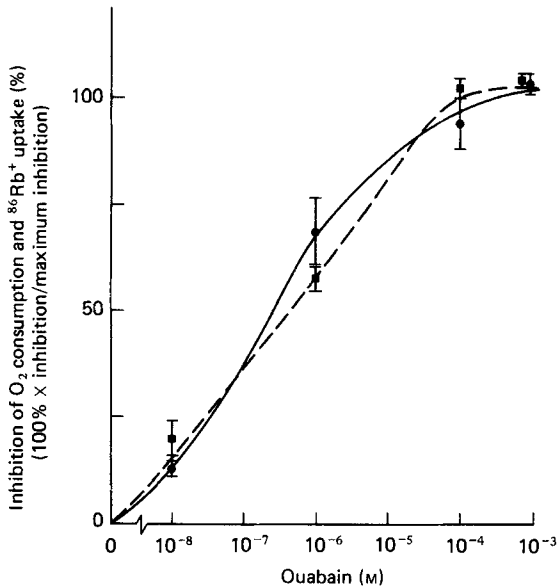


Fig. 1. Inhibition of lamb hepatocyte oxygen consumption (●—●, $n = 3$) and $^{86}\text{Rb}^+$ uptake (■—■, $n = 2$) by ouabain. Inhibitions are expressed as a percentage of maximum inhibition. Values are means with their standard errors represented by vertical bars.

for adult ewes in Expt 1 were used for hepatocyte isolation. O_2 consumption, ouabain-sensitive respiration (1×10^{-4} M-ouabain) and ouabain-insensitive respiration rates were measured for hepatocytes isolated from the fed and starved sheep as described in Expt 1. Similarly, the ouabain-sensitive $^{86}\text{Rb}^+$ uptake measurement was made for hepatocytes isolated from two fed and two starved sheep, as outlined in Expt 1.

Analysis of results. Respiration rates and $^{86}\text{Rb}^+$ uptakes were expressed on a tissue dry-weight basis, determined after 12 h at 90° . All values were analysed by analysis of variance and the treatment means were compared ($P < 0.05$) by either t tests or by Student-Newman-Keul's multiple-range tests (Steel & Torrie, 1960).

RESULTS

Expt 1

Ouabain inhibition of O_2 and $^{86}\text{Rb}^+$ uptake. The response curves for ouabain-inhibition of O_2 and $^{86}\text{Rb}^+$ uptake by lamb hepatocytes are shown in Fig. 1.

The nature of ouabain inhibition of both O_2 and $^{86}\text{Rb}^+$ uptakes was similar. The response curves for these measurements were sigmoidal (Fig. 1). There was greater inhibition of both uptakes by 10^{-3} and 10^{-4} M-ouabain than by an ouabain concentration of 10^{-6} M, or less.

Ouabain inhibition of $^{86}\text{Rb}^+$ uptake in lamb hepatocytes was immediate (Fig. 2). Within 1 min of exposure to 10^{-4} M-ouabain, 48.2 (SE 4.6)% of the $^{86}\text{Rb}^+$ uptake by hepatocytes had been inhibited and at 5 min, 68.1 (SE 0.9)% of $^{86}\text{Rb}^+$ uptake were inhibited. The latter value was not significantly different ($P > 0.05$) from the maximum inhibition of 80.5 (SE 1.9)% attained at 30 min. Peak inhibition of $^{86}\text{Rb}^+$ uptake by ouabain, expressed as a percentage of total $^{86}\text{Rb}^+$ uptake, was maintained from 5 to 60 min of incubation (Fig. 2).

O_2 uptake and ouabain-sensitive respiration. The results of the effect of lactation on respiration indices and milk production are shown in Table 1. Total milk production

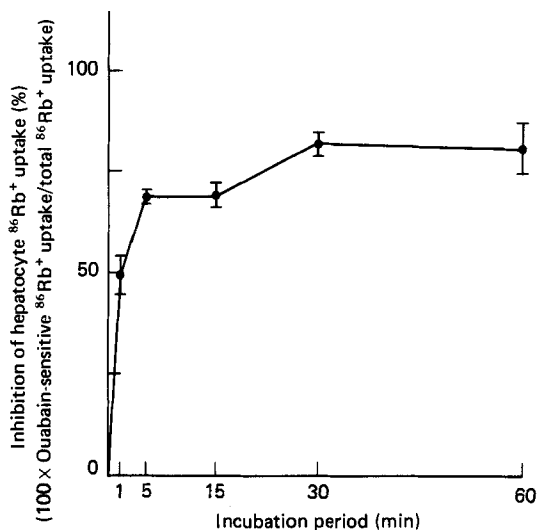


Fig. 2. Time-course of inhibition of lamb hepatocyte $^{86}\text{Rb}^+$ uptake by ouabain (1×10^{-4} M). Mean values of duplicate observations are plotted with their standard errors represented by vertical bars.

dropped ($P < 0.05$) by 42% from week 4 to week 8 of lactation. Total O_2 consumption rates of the liver biopsies were not significantly different ($P > 0.05$) between non-lactating and lactating ewes (Table 1). During peak lactation, the ouabain-sensitive respiration of the liver biopsies accounted for 45% of the total tissue O_2 consumption. This percentage was 1.24–1.37 times those measured during late lactation and during the dry period. However, the magnitude of ouabain-sensitive respiration in liver biopsies of ewes at peak lactation (1.48 (SE 0.15) nmol O_2 /mg per min) was not significantly higher ($P < 0.05$) than similar measurements made during late lactation and during the non-lactating period. Throughout lactation and during the dry period the magnitude of ouabain-insensitive respiration did not change.

Viabilities of the hepatocyte preparation from the mature sheep were 11% lower ($P < 0.05$) than measurements of 90–93% determined for lamb hepatocytes (Table 2). The magnitude of this difference was much less than the differences of 40–70% in respiration measurements between adults and lambs. Previous work from our laboratory has shown that significant depressions in respiration values due to the preparation may be evident in hepatocyte preparations of less than 50% viability (McBride & Milligan, 1985). Lamb hepatocyte preparations with viability of less than 50% exhibited significantly lower total and ouabain-sensitive respiration than lamb hepatocyte preparations of greater than 90% viability. The magnitude of total and ouabain-sensitive respiration was not determined for lamb hepatocyte preparations having viabilities between 50 and 80%. However, it should be noted that the lower total and ouabain-sensitive respiration of the hepatocytes isolated from mature sheep may partially reflect the lower viabilities of these cell preparations.

The O_2 consumption rates of hepatocytes from mature sheep were 39–46% lower ($P < 0.05$) than the values of 4.86–5.62 nmol O_2 /mg per min observed for cells from lambs (Table 2). Additionally, the magnitude of ouabain-sensitive respiration of hepatocytes isolated from mature sheep was 67–69% lower ($P < 0.05$) than similar measurements for lamb hepatocytes. The only respiration index of the sheep hepatocytes that did not differ ($P > 0.05$) between adults and lambs was ouabain-insensitive respiration (Table 2).

Table 1. *Milk production of lactating ewes and oxygen consumption indices from liver biopsies of lactating and non-lactating ewes*
(Mean values with their standard errors)

Physiological state	n	Milk production (g/d)		Total O ₂ consumption (nmol O ₂ /mg† per min)		Percentage inhibition of O ₂ consumption by ouabain		Ouabain-sensitive respiration (nmol O ₂ /mg† per min)		Ouabain-insensitive respiration (nmol O ₂ /mg† per min)	
		Mean*	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Lactating											
4 weeks	10	2252 ^a	252	3.36 ^a	0.31	45.1 ^a	3.8	1.48 ^a	0.15	1.88 ^a	0.25
8 weeks	9	1305 ^b	198	3.16 ^a	0.27	32.9 ^b	4.2	1.04 ^a	0.12	2.12 ^a	0.26
Non-lactating	9			3.14 ^a	0.43	36.5 ^b	3.0	1.15 ^a	0.20	1.99 ^a	0.28

^{a, b} Means within columns with different superscript letters were significantly different ($P < 0.05$).

* Values are means of five observations per treatment.

† Dry tissue.

Table 2. *Growth rates of lambs and oxygen consumption indices of hepatocytes isolated from mature sheep and infant lambs*
(Mean values with their standard errors)

Age	n	Average daily gain (g/d)		Hepatocyte viability (%)		Total O ₂ consumption (nmol O ₂ /mg* per min)		Percentage inhibition of O ₂ consumption by ouabain		Ouabain-sensitive respiration (nmol O ₂ /mg* per min)		Ouabain-insensitive respiration (nmol O ₂ /mg* per min)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
4 weeks	5	285 ^a	40	93 ^a	2	5.62 ^a	0.28	47.8 ^a	3.8	2.67 ^a	0.21	2.95 ^a	0.31
8 weeks	4	230 ^a	41	90 ^a	2	4.86 ^a	0.22	51.0 ^a	3.0	2.50 ^a	0.19	2.36 ^a	0.17
3 years	2	—	—	80 ^b	1	3.00 ^b	0.10	27.9 ^b	2.6	0.82 ^b	0.07	2.18 ^a	0.14

^{a, b} Means within columns with different superscript letters were significantly different ($P < 0.05$).

* Dry cells.

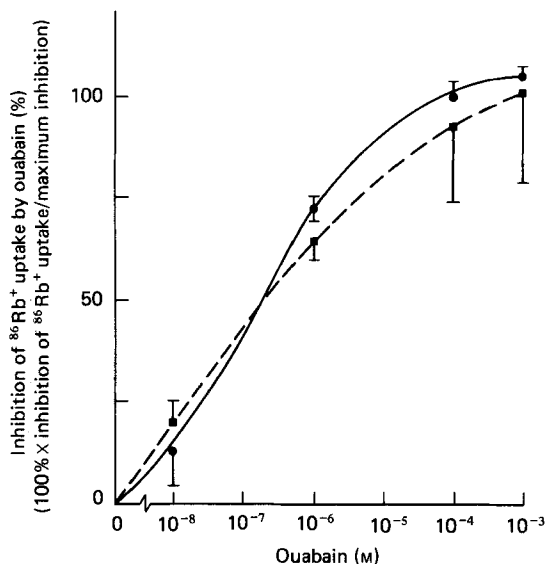


Fig. 3. Inhibition of hepatocyte $^{86}\text{Rb}^+$ uptake by ouabain. The response curves for fed (●—●) and starved (■—■) sheep. Mean values of four observations per treatment are plotted with their standard errors represented by vertical bars.

Ouabain-sensitive respiration accounted for 48–51% of the total O_2 consumption of hepatocytes from young growing (230–285 g/d) lambs but decreased ($P < 0.05$) to only 28% of the O_2 uptake of hepatocytes from mature animals held at maintenance. There were no differences ($P > 0.05$) in respiration measurements between hepatocytes from lambs of 4 weeks of age and those from lambs of 8 weeks of age.

Expt 2

$^{86}\text{Rb}^+$ uptake measurements. The dose–response curves of ouabain-inhibition of $^{86}\text{Rb}^+$ uptake of hepatocytes from fed and starved sheep are shown in Fig. 3. The sigmoidal pattern of ouabain inhibition of hepatocyte uptake of $^{86}\text{Rb}^+$ was similar for both fed and starved sheep and similar to the response curve observed for lambs. The time-course of ouabain inhibition of $^{86}\text{Rb}^+$ uptake by hepatocytes from fed and starved sheep is shown in Fig. 4. The magnitude of ouabain-sensitive $^{86}\text{Rb}^+$ uptake by hepatocytes was similar ($P > 0.05$) for both groups of sheep for 1 and 5 min of incubation. At 15, 30 and 60 min of exposure to ouabain, ouabain-sensitive $^{86}\text{Rb}^+$ uptake by hepatocytes from fed sheep was three to six times greater ($P < 0.05$) than by cells from starved sheep (Fig. 4). As found with lamb hepatocytes, maximum percentages of ouabain inhibition of hepatocyte intakes of $^{86}\text{Rb}^+$ were attained within 5 min of exposure to 10^{-4} M-ouabain.

Whole-animal and hepatocyte respiration. Whole-animal O_2 consumption rates and hepatocyte respiration rates are shown in Table 3. Whole-animal O_2 consumption rate was reduced ($P < 0.05$) 10% by starvation. Although the rate of O_2 uptake by hepatocytes from starved sheep was 29% less than that of cells from fed sheep, the values were not significantly different. Viabilities of the hepatocyte preparations were in excess of 90% and did not differ ($P > 0.05$) with feed-intake treatment (Table 3).

Ouabain-sensitive respiration accounted for 17.8 and 41.1% of the total O_2 consumption of hepatocytes isolated from starved and fed sheep respectively. Ouabain-sensitive O_2 uptake of hepatocytes from starved sheep was 0.48 nmol/mg dry cell weight per min or 62% lower

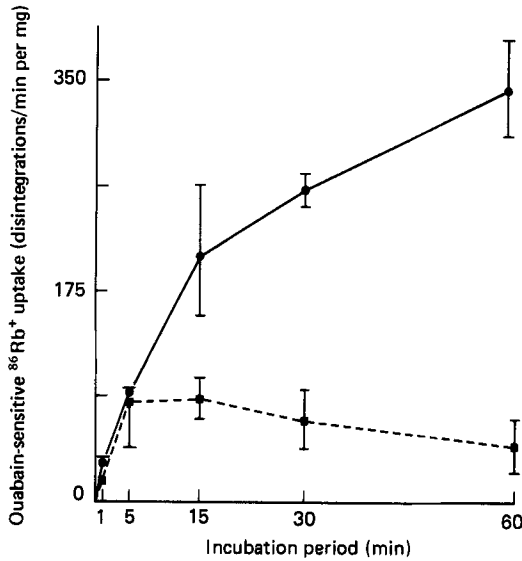


Fig. 4. Time-scale of ouabain-sensitive $^{86}\text{Rb}^+$ uptake of hepatocytes isolated from fed (●—●) and starved (■---■) sheep. Mean values of four observations per treatment are plotted with their standard errors represented by vertical bars and are expressed on a dry-tissue-weight basis.

($P < 0.05$) than for hepatocytes from fed sheep. The ouabain-insensitive component of hepatocyte O_2 consumption did not differ ($P > 0.05$) between preparations from fed and starved sheep (Table 3).

DISCUSSION

That ouabain-sensitive respiration rates are an estimate of the magnitude of Na^+, K^+ -ATPase-dependent respiration relies on the accepted high degree of specificity of ouabain as an inhibitor of Na^+, K^+ -ATPase. Inhibition of Na^+, K^+ -ATPase was confirmed by demonstration of inhibition of tissue uptake of $^{86}\text{Rb}^+$. On the other hand, the respiration that is not sensitive to inhibition by ouabain provides for cellular energy expenditures other than energy costs of Na^+, K^+ -transport including, for example, the energy cost of cellular syntheses. In the present study, lactational state, age and feeding level had no significant effect on the magnitude of ouabain-insensitive respiration of liver biopsies and isolated hepatocytes. That is, the aggregate energy expenditure on processes other than Na^+, K^+ -transport did not change, although it is entirely conceivable that the magnitude of individual components may have changed with physiological state.

During peak lactation, ouabain-sensitive respiration of liver biopsies of ewes accounted for 45% of the total tissue O_2 consumption. This percentage corresponded with the highest magnitude of ouabain-sensitive respiration in the liver of lactating ewes. It might be expected that elevated Na^+, K^+ -ATPase activity could occur in the liver to support active uptake of substrates, however, Van Dyke *et al.* (1983) found that less than 3% of Na^+, K^+ -ATPase-dependent respiration of perfused rat liver was linked to the uptake of organic anions. Thus our results indicate that the maintenance energy costs required to support ion transport in the liver may increase during peak lactation. Additionally, higher ouabain-sensitive respiration has been found in skeletal muscle and duodenal mucosa of lactating animals compared with values obtained for tissues from non-lactating animals

Table 3. Whole animal consumption, hepatocyte-total O₂ consumption, ouabain-sensitive and ouabain-insensitive respiration and percentage inhibition of hepatocyte O₂ consumption by ouabain in starved and fed sheep

Physiological state	n	Whole animal O ₂ consumption (ml O ₂ /kg per h)		Hepatocyte total O ₂ consumption (nmol O ₂ /mg* per min)		Percentage inhibition of O ₂ consumption by ouabain		Ouabain-sensitive respiration (nmol O ₂ /mg* per min)		Ouabain-insensitive respiration (nmol O ₂ /mg* per min)		Hepatocyte viability (%)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fed	4	273 ^a	5	3.02 ^a	0.18	41.1 ^a	4.1	1.27 ^a	0.15	1.75 ^a	0.10	90.5 ^a	2.3
Starved	4	245 ^b	3	2.13 ^a	0.44	17.8 ^b	7.1	0.48 ^b	0.20	1.65 ^a	0.26	97.8 ^a	2.3

^{a, b} Means within columns with different superscript letters were significantly different ($P < 0.05$).

* Dry cells.

(Gregg & Milligan, 1982*c*; McBride & Milligan, 1984). Thus elevation of cellular cation transport may be prevalent within a number of tissues in lactating animals.

Total O₂ consumption rates of the lamb hepatocytes of the present study were lower than those previously reported for hepatocytes from lambs of a similar age (Clark *et al.* 1976). However, these previous workers used an O₂-saturated buffer for their O₂-uptake measurements. In our study, an air-saturated buffer was used in all O₂ consumption measurements. Studies using adult rat hepatocytes have shown higher O₂ uptake rates in O₂-saturated buffers (11.3–11.4 nmol O₂/mg dry weight per min; Ismail-Beigi *et al.* 1979; Clark *et al.* 1982) compared with values obtained for rat hepatocytes incubated in air-saturated buffers (6.62–9.48 nmol O₂/mg dry weight per min; Van Dyke *et al.* 1983). Furthermore, the O₂ consumption rates measured for lamb hepatocytes in the present study were within the range of O₂ uptake rates determined for lamb liver *in situ* (4.74–10.78 nmol O₂/mg dry weight per min; Edelstone & Holzman, 1981).

Ouabain-sensitive respiration of lamb hepatocytes accounted for approximately 50% of total hepatocytes isolated from mature sheep. Similarly, total hepatocyte O₂ consumption was 62–87% greater for growing lambs than for mature sheep. The increased ouabain-sensitive component of respiration of lamb hepatocytes accounted for 71–90% of the difference between their rate of O₂ consumption and the lower rate of uptake by hepatocytes from mature sheep. Other work has shown that the number and activity of Na⁺,K⁺-ATPase units are greater in the liver of young animals compared with those found in older animals (Lin *et al.* 1979*a, b*). Thus higher maintenance energy expenditures would seemingly be necessary to support Na⁺,K⁺-ATPase activity in hepatocytes of growing lambs compared with mature sheep. This suggestion is consistent with the observation that mature animals have a lower overall maintenance energy expenditure than young growing animals (Webster, 1981) although admittedly the liver may only account for 10–15% of the total heat production of animals (Lautt, 1976, 1977; Edelstone & Holzman, 1981).

Elevation of Na⁺,K⁺-ATPase activity appears to be an important event in both hypertrophic and hyperplastic growth. For example, elevation of Na⁺,K⁺-ATPase activity is one of the initiating events associated with stretch-induced protein synthesis in embryonic skeletal muscle cells (Vandenburgh & Kaufman, 1981). Furthermore, in cultured mammalian cell lines, activation of Na⁺,K⁺-ATPase is an early and essential step in DNA synthesis and cell proliferation in response to mitogenic stimulation (Kaplan, 1978; Rozengurt & Mendoza, 1980; Mummery *et al.* 1981). It is, therefore, possible that the enhanced Na⁺,K⁺-transport in the livers of growing lambs and lactating ewes, as contrasted to adult sheep and dry ewes, is directly associated with the synthetic activities involved in growth and lactation.

It is also apparent from the present study that the magnitude of ouabain-sensitive respiration of sheep hepatocytes changes in relation to the feeding level of the animal. Hepatocytes from sheep fed to maintenance expended 2.6-fold more energy in support of Na⁺,K⁺-transport than those from starved sheep. Background or maintenance energy expenditure on hepatic ion transport, therefore, is likely not to be constant in animals at different feeding levels. Furthermore, reduced energy expenditure on Na⁺,K⁺-transport could be of survival value during intervals of food deprivation.

The higher levels of ouabain-sensitive respiration were probably a result of increased numbers of Na⁺,K⁺-ATPase units, or increased activity of existing enzyme, or a combination of both. Measurements were not made that would yield an answer to these possibilities. However, it is of interest to note that the dose-response curve for ouabain inhibition of the enzyme (as measured by ⁸⁶Rb⁺ uptake) was not altered by starvation. Previously, it was reported (Gregg & Milligan, 1982*a*) that although cold exposure of sheep caused increased ouabain-dependent respiration of skeletal muscle, the dose-response curve for ouabain

inhibition of respiration of the tissue was unchanged. These results suggest that the tissue affinity for ouabain was not influenced by the animal's physiological state.

The requirement for energy in the transport of Na^+ and K^+ across the plasma membrane of cells in the liver of sheep accounts for 28–41% of the O_2 uptake of this tissue incubated in vitro with energy substrates. Energy expenditure on Na^+ , K^+ -transport is substantially influenced by the physiological state of the animal from which the tissue was taken. Thus, hepatic Na^+ , K^+ -transport appears to be a metabolic component of maintenance energy expenditure that is not a constant function of body-weight.

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