Influence of feed intake and starvation on the magnitude of Na^+,K^+-ATPase(*EC* 3.6.1.3)-dependent respiration in duodenal mucosa of sheep

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1. Oxygen consumption and Na^+,K^+-ATPase(*EC* 3.6.1.3)-dependent (ouabain-sensitive) and -independent respiration were measured for duodenal mucosa biopsies from 10-month-old sheep given two levels of digestible energy (DE) intake (7·6–7·7 and 14·8 MJ lucerne (*Medicago sativa*) pellets/d) and following 48 h of starvation.

2. The mucosal biopsies were determined to be structurally intact and free of adherent bacteria on histological and scanning-electron-microscope examinations.

3. The use of D-glucose as a substrate during incubations did not elevate ($P > 0·05$) the respiration indices of the biopsies over those measured during acetate incubations.

4. Glucose uptake did not ($P > 0·05$) influence the Na^+,K^+-ATPase-dependent respiration of the mucosal biopsies.

5. Na^+,K^+-ATPase-dependent respiration accounted for 50% of the total O_2 consumption of the mucosal biopsies of sheep given the lower level of DE.

6. Total O_2 consumption of the duodenal mucosa was not ($P > 0·05$) increased when sheep were given the higher level of DE but Na^+,K^+-ATPase-dependent respiration of the mucosa was elevated ($P < 0·01$) by 37% during this period.

7. When sheep were starved for 48 h, total O_2 consumption of the mucosal biopsies was not ($P > 0·05$) affected, however, Na^+,K^+-ATPase-dependent respiration of the biopsies dropped ($P < 0·01$) by 45%.

8. Na^+,K^+-ATPase-dependent respiration accounted for 61·3% of the O_2 uptakes of mucosa from the sheep given the higher level of DE and 28·3% of the O_2 uptake of mucosa from fasted sheep.

Na^+,K^+-ATPase (*EC* 3.6.1.3)-dependent respiration is a major component of in vitro cellular energy expenditure of skeletal muscle, brain, liver, intestine and kidney (Ismail-Beigi & Edelman, 1971; Asano *et al.* 1976; Liberman *et al.* 1979). The magnitude of the Na^+,K^+-ATPase response appears to be related to the function of the tissue (Ismail-Beigi & Edelman, 1970; Mandel & Balaban, 1981). In mammalian kidney, energy expenditure by Na^+,K^+-ATPase-mediated Na^+ reabsorption accounts for up to 70% of total oxygen uptake of the organ (Balaban *et al.* 1980). In contrast, mammalian liver Na^+,K^+-ATPase activity is not tightly coupled to active absorption and, therefore, it accounts for only one-third of the total O_2 consumption of the tissue (Van Dyke *et al.* 1983). The level of Na^+,K^+-ATPase-dependent respiration in tissues also appears to be related to the physiological status of the animal. Hyperthyroid animals usually exhibit elevated Na^+,K^+-ATPase activity in various tissues including muscle, kidney and liver (Ismail-Beigi & Edelman, 1970; Asano *et al.* 1976; Ismail-Beigi *et al.* 1979). Higher Na^+,K^+-ATPase-dependent respiration is also induced in skeletal muscle of animals exhibiting elevated metabolic rates due to the physiological stresses of cold (Gregg & Milligan, 1982*a*) and lactation (Gregg & Milligan, 1982*c*).

The gastrointestinal (GI) tract has been proposed as a major site of both heat production (Webster, 1981) and Na^+,K^+-ATPase activity in the body (Liberman *et al.* 1979). Therefore,

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the purposes of the present study were to determine the magnitude of in vitro energy expenditure of sheep duodenal mucosa on Na^+,K^+-transport and the effects of changes in the physiological state or level of feed intake on such expenditure.

EXPERIMENTAL

Animals

Five 10-month-old Suffolk wethers were fitted with cannulas in the proximal portion of the descending duodenum, as described by McBride *et al.* (1983). Experimentation with the animals commenced 2 months following cannulation to allow for full recovery of the intestinal mucosa. The animals were individually fed twice daily (08.00 and 16.00 hours) equal amounts of ground, pelleted lucerne (*Medicago scetiva*) to achieve digestible energy (DE) intakes of 7·6 (SE 0·2) MJ/d (LE1) then 14·8 (SE 0·5) MJ/d (HE) and finally 7·7 (SE 0·3) MJ/d (LE2). After readjustment to the lower DE intake, the sheep were fasted for 48 h. The animals were given the different energy intake levels for 3–4 weeks before mucosal biopsy sampling. Throughout the study, water and salt were offered *ad lib.*

Mucosal biopsies were excised from the descending duodenum of the sheep using a Quinton suction biopsy device as described by McBride *et al.* (1983). A single biopsy was taken 1–2 h following morning feeding on two consecutive days during each period of the energy intake regimens. For the animals fasted for 48 h, two biopsies were taken from each animal before the measurement of whole animal O_2 consumption.

Whole animal respiration

Whole animal respiration rates were determined for each animal during each DE intake level and following the 48 h fast. Oxygen consumption was measured for a 12 h period by respiratory gaseous exchange as described by Young *et al.* (1975).

Effect of ouabain concentration on O_2 consumption and $^{86}Rb^+$ uptakes. Preliminary trials were conducted to obtain dose-response curves for the effect of ouabain on the inhibition of mucosal O_2 consumption and $^{86}Rb^+$ uptake. The results of these preliminary trials, described later, indicated that the minimum concentration required to inhibit Na^+,K^+-ATPase activity completely was $2{\cdot}0 \times 10^{-5}$ M-ouabain. This dosage of ouabain was used in measurements made during the main experiment.

Excised biopsies from a single sheep fed on the lower DE level intake were washed in a modified Krebs–Henseleit buffer (KHB) (Dawson *et al.* 1969) containing 10 mM-D-glucose or 5 mM-acetate, and 20 mM-Hepes. The samples were pre-incubated for 10 min in the same air-saturated buffer (180 nmol O_2/ml, 700 mmHg; Umbreit *et al.* 1964) maintained at pH 7·4 (SE 0·1) and 37°. Rates of O_2 uptake of the biopsies were measured polarographically using a YSI model 53 O_2 electrode assembly. Initial O_2 uptakes of the biopsies were measured for 15 min, then the biopsies were transferred to another electrode chamber containing the KHB with ouabain concentrations of 0, 10^{-9}, 10^{-7}, 10^{-5} or 10^{-3} M. Uptakes of O_2 by these biopsies were measured for a further 40–45 min, during which time the O_2 consumption rates remained linear. The maximum reduction in the rate of O_2 consumption by the ouabain-treated samples was considered to be a measure of Na^+,K^+-ATPase-dependent respiration. Mean percentage inhibition of O_2 uptake by ouabain was calculated for each ouabain concentration and a dose-response curve was constructed expressing inhibition as a percentage of maximum inhibition.

Another sheep, given the lower level of DE was slaughtered and a segment (approximately 200 mm) of the descending duodenum was removed. The lumen was rinsed with ice-cold phosphate-buffered saline (10 mM-sodium phosphate, 0·14 M-sodium chloride, pH 7·4). The intestinal segment was stripped of the serosa (Liberman *et al.* 1979) and the mucosa cut

into pieces (5 mm diameter) with a cork borer. These were weighed and transferred to the incubation buffer. The uptake of $^{86}Rb^+$ by the duodenal mucosa was measured in 2 ml KHB (pH 7·4, 37°) containing bovine serum albumin (20 g/l), 5 μCi $^{86}Rb^+$, 0·1 mM-RbCl and 10 mM-D-glucose in an atmosphere of air. Inhibition of $^{86}Rb^+$ uptake by ouabain during 10 min of incubation (37°), was determined in triplicate for each concentration of ouabain. An ouabain dose-response curve was constructed by expressing inhibition of $^{86}Rb^+$ uptake as a percentage of maximum inhibition of $^{86}Rb^+$ uptake at each ouabain concentration. The time-course of 10^{-4} M-ouabain inhibition of $^{86}Rb^+$ uptake by the mucosa biopsies was determined in triplicate for incubation periods of 1, 5, 15 and 60 min.

On completion of the various incubations, $^{86}Rb^+$ uptake was stopped by aspiration of the medium and the mucosal samples were rinsed with ice-cold phosphate-buffered saline. The tissues were transferred to 15-ml plastic scintillation vials and 1 ml Protosol (New England Nuclear, Boston, MA.) was added to the samples. The samples were subsequently digested for 1 h at 55° in a shaking water-bath. Glacial acetic acid (50 μl) was added to decolourize the dissolved samples; 10 ml Unisolve 1 (Tetrochem Laboratories Ltd, Edmonton, Alberta) was added to the vials and the samples were immediately counted in a Nuclear Chicago Mark I scintillation counter using balance-point counting with a 20:1 dynamic range window. All measurements were expressed as disintegrations/min per mg dry weight. The wet weight of each sample was converted to a dry-weight basis using the dry matter content of 145 (SE 15) g/kg determined for identical mucosal samples.

Measurements of mucosal respiration. Measurements of the respiration of biopsies excised from the sheep given different amounts of DE were conducted as described in the preceding section with the modification that Na^+,K^+-ATPase-dependent respiration was determined from samples incubated in KHB containing $2·0 \times 10^{-5}$ M-ouabain or devoid of Na^+. In the Na^+-free KHB, NaCl was replaced on an equivalent molar basis with choline chloride. The respiration rates of the mucosal biopsies were expressed on a biopsy dry-weight basis. Following O_2 consumption measurements, biopsies were dried at 90° for 12 h for dry-weight determinations.

Morphology of the duodenal mucosal biopsies

To determine which anatomical portions of the intestinal wall had been removed by the biopsy procedure and to verify that the structural integrity of the intestinal villi had been maintained, histological sections were prepared from each animal at all energy intake levels. The samples were preserved, dehydrated, sectioned and stained according to the procedures of Perera *et al.* (1975).

Scanning electron micrographs were prepared for the mucosal biopsies to assess surface morphology and to determine the extent of gut bacteria colonization on the duodenal epithelium. The excised biopsies were washed with phosphate buffer (63·2 mM-$Na_2HPO_4 . 7H_2O$, 15·0 mM-$NaHPO_4 . H_2O$; pH 7·4) and were fixed with phosphate-buffer solvents. The samples were mounted on nylon mesh (Perera *et al.* 1975) fixed in glutaraldehyde (10 g/l) for 24 h at 4° then post-fixed in osmium tetroxide (10 g/l) for 2 h. The samples were subsequently dehydrated in a graded ethanol series and were taken to amyl acetate before critical-point drying in carbon dioxide (Anderson, 1951). The biopsies were coated with gold-palladium and were then scanned on a Cambridge Stereoscan 180 scanning electron microscope at a voltage of 20 kV.

Analysis of results

Values were subjected to analysis of variance and the treatment means were compared by either *t* tests or by Student-Newman-Keul's multiple range tests (Steel & Torrie, 1960).

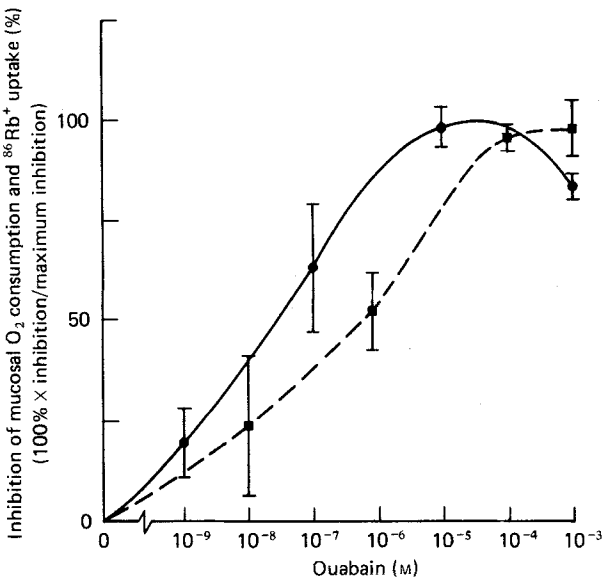

Fig. 1. Inhibition of ouabain of respiration (——) and $^{86}Rb^+$ uptake (----) by duodenal mucosa of sheep given 7·6 MJ digestible energy/d. Values are means with their standard errors represented by vertical bars. The mucosal oxygen consumption rates in the absence of ouabain and when maximally inhibited by ouabain were 5·85 (SE 0·29) and 3·20 (SE 0·16) nmol O_2/mg per min respectively.

RESULTS

Morphology of the mucosal biopsies

The histological and fine structure of the duodenal mucosal biopsies are shown in Plates 1 (*a–c*). The biopsy procedure yielded an intact mucosal preparation devoid of the serosa and the longitudinal and transverse layers of the muscularis externa (Plate 1 (*a*)). The structural integrity of the duodenal mucosa and the abundance of the intestinal villi did not appear to change with a change in energy intake. Plates 1 (*b* and *c*) are scanning electron micrographs of a villus from a duodenal mucosal biopsy excised from a sheep fed at maintenance. Individual epithelial cells appear as polygonal structures covering the villus (Plate 1 (*b*)). The preparation was relatively devoid of mucous and no bacteria were found adhering to the epithelium. At high magnifications, dense uniform microvilli are seen to cover the surface of the epithelial cells (Plate 1 (*c*)). Similar morphological features were present in mucosal biopsies excised from animals given the other energy intake levels.

Dose-response curves and time-scale of ouabain inhibition

The dose-response curves for ouabain inhibition of O_2 and $^{86}Rb^+$ uptakes are shown in Fig. 1. The shapes of the ouabain dose-response curves were sigmoidal for both measurements. The lowest concentration of ouabain yielding maximum inhibition of O_2 uptake was 10^{-5} M, while there was greater inhibition of $^{86}Rb^+$ uptake at 10^{-4} M- than at 10^{-6} M-ouabain measurements respectively.

The time-course of ouabain inhibition of $^{86}Rb^+$ uptake is shown in Fig. 2. Maximum inhibition of $^{86}Rb^+$ uptake by the mucosal biopsies was reached within 5 min of exposure to 10^{-4} M-ouabain. However, ouabain inhibition at 1 min was not statistically different ($P > 0·05$) from the maximum inhibition values. Ouabain inhibition of $^{86}Rb^+$ uptake by duodenal mucosa occurred rapidly, reaching a maximum within 5 min of exposure of ouabain to the tissue.

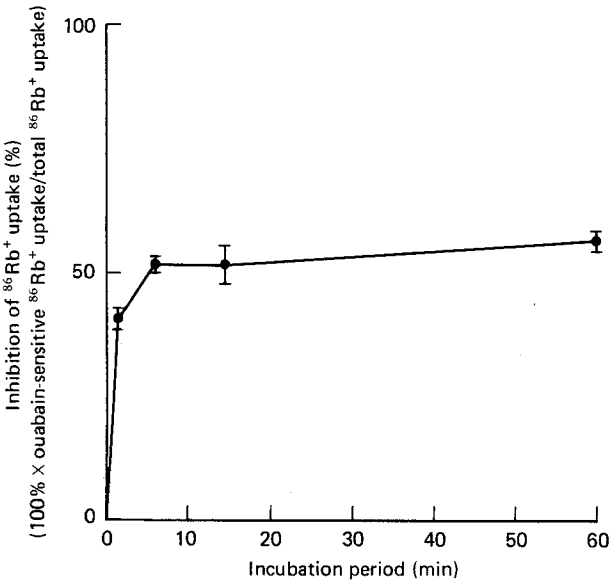

Fig. 2. Time-scale of ouabain inhibition of $^{86}Rb^+$ uptake by duodenal mucosa of sheep given 7·6 MJ digestible energy/d. Values are means with their standard errors represented by vertical bars.

O_2 uptake and Na^+,K^+ ATPase-dependent respiration

Total O_2 uptake and ouabain-sensitive respiration of duodenal mucosa are shown in Tables 1 and 2. The use of acetate as a substrate instead of glucose had no significant ($P > 0.05$) effect on total O_2 consumption of duodenal mucosa (Table 1). Total O_2 consumption, Na^+,K^+-ATPase-dependent respiration and Na^+,K^+-ATPase-independent respiration and ouabain inhibition values measured for mucosal biopsies of sheep given 7·6 MJ DE/d, were similar when measured 3 months later after the animals had been returned to a similar DE intake of 7·7 MJ/d (Table 1).

The Na^+,K^+-ATPase-dependent respiration of duodenal mucosa of sheep given 7·6 MJ DE/d was also assessed in a Na^+-free medium (Table 1). The estimates of Na^+,K^+-ATPase-dependent respiration, derived from the use of Na^+-free media, were not significantly different ($P > 0.05$) from the values for Na^+,K^+-ATPase-dependent respiration using ouabain as an inhibitor of Na^+,K^+-ATPase. Furthermore, the total O_2 consumption and Na^+,K^+-ATPase-independent respiration were similar for biopsies in which Na^+,K^+-ATPase-dependent respiration was estimated with the use of Na^+-free media and those subjected to ouabain inhibition. The extent of inhibition of respiration by ouabain and by an absence of Na^+ did not differ significantly ($P > 0.05$).

The DE intake of the sheep given the HE diet was approximately twice that received by the sheep during maintenance feeding (Table 2). Feeding level was reflected by the O_2 consumption rates of the animals (Table 2). The O_2 consumption of sheep given 7·6 MJ DE/d was 37% higher ($P > 0.01$) than that when fasted. At the higher (14·8 MJ/d) DE intake, whole animal O_2 consumption rates of the sheep were 20% greater ($P < 0.01$) than at the lower (7·6 MJ/d) DE intake and 99% higher ($P < 0.01$) than the O_2 consumption rates measured during fasting. The total O_2 uptake of the mucosal biopsies did not ($P > 0.05$) differ with level of DE intake. However, the Na^+,K^+-ATPase-dependent respiration of the duodenal mucosa was influenced ($P < 0.01$) by level of DE intake. Na^+,K^+,-ATPase-dependent respiration of mucosal biopsies dropped 45% from LE levels

Table 1. *Total oxygen consumption, percentage inhibition and Na^+,K^+-ATPase* (EC *3.6.1.3*)*-dependent*† *and -independent*‡ *respiration of duodenal mucosa of sheep given 7·6 MJ digestible energy (DE) /d*

(Mean values with their standard errors for ten observations)

		DE intake (MJ/d)		Total O_2 consumption (nmol O_2/mg§ per min)		Percentage inhibition of O_2 consumption		Na^+,K^+-ATPase-dependent respiration (nmol O_2/mg§ per min)		Na^+,K^+-ATPase-independent respiration (nmol O_2/mg§ per min)	
Treatment	Substrate	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
LE1	Acetate	7·6	0·2	5·32	0·28	50·1	4·6	2·68	0·30	2·64	0·30
	Glucose			5·65	0·41	48·1	2·6	2·69	0·20	2·96	0·33
	Na^+-free			5·57	0·43	45·5	2·8	2·53	0·22	3·04	0·13
LE2	Glucose	7·7	0·3	5·84	0·84	47·1	4·3	2·72	0·44	3·12	0·67

LE1 and LE2 refer to the level of DE intake.
Means within columns were not significantly different ($P > 0·05$).
† Na^+,K^+-ATPase-dependent respiration = total O_2 consumption × inhibition of O_2 consumption by ouabain (or inhibition due to Na^+-free buffer).
‡ Na^+,K^+-ATPase-independent respiration = total O_2 consumption − Na^+,K^+-ATPase-dependent respiration.
§ Dry tissue weight.

Table 2. *Whole animal and mucosal oxygen consumption, percentage inhibition and Na^+,K^+-ATPase* (EC *3.6.1.3*)*-dependent† and -independent‡ respiration of duodenal mucosa of sheep given two levels of digestible energy (DE) intake or starved for 48 h*

(Mean values with their standard errors for ten observations unless otherwise stated)

	DE intake (MJ/d)		Whole animal O_2 consumption (ml O_2/kg per h)		Mucosal O_2 consumption (nmol O_2/mg§ per min)		Percentage inhibition of O_2 consumption		Na^+,K^+-ATPase-dependent respiration (nmol O_2/mg§ per min)		Na^+,K^+-ATPase-independent respiration (nmol O_2/mg§ per min)	
Treatment	Mean‖	SE	Mean‖	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Starvation	$0{\cdot}0^a$	0·0	206^a	11	5·21	0·23	$28{\cdot}6^a$	2·7	$1{\cdot}48^a$	0·14	$3{\cdot}73^A$	0·25
LE1	$7{\cdot}6^b$	0·2	328^b	11	5·65	0·41	$48{\cdot}1^b$	2·6	$2{\cdot}69^b$	0·20	$2{\cdot}96^B$	0·33
HE	$14{\cdot}8^c$	0·5	409^c	14	6·07	0·39	$61{\cdot}3^c$	3·3	$3{\cdot}69^c$	0·25	$2{\cdot}38^B$	0·18

LE1 and HE refer to the level of DE intake.

^{a,b,c} Means within a column with different superscript letters were significantly different ($P < 0{\cdot}01$).

^{A,B} Means within this column with different superscript letters were significantly different ($P < 0{\cdot}05$).

† Na^+,K^+-ATPase-dependent respiration = total O_2 consumption × inhibition of O_2 consumption by ouabain.

‡ Na^+,K^+-ATPase-independent respiration = total O_2 consumption − Na^+,K^+-ATPase-dependent respiration.

§ Dry tissue weight

‖ Mean values for five observations.

when the sheep were fasted for 48 h. Similarly, Na^+,K^+-ATPase-dependent respiration of the duodenal mucosa increased by 37% from LE levels when the sheep were fed at the HE level. The changes in Na^+,K^+-ATPase-dependent respiration significantly ($P < 0.01$) affected the percentage inhibition of total respiration by ouabain with an increase from 28·6 to 61·3% in going from fasting to feeding well-above maintenance (Table 2).

Na^+,K^+-ATPase-independent respiration of mucosal biopsies was 26–57% higher ($P < 0.01$) in fasted sheep compared with fed sheep (Table 2). Na^+,K^+-ATPase-independent respiration of mucosal biopsies was not significantly different in sheep given either 7·6 MJ DE/d or 14·8 MJ DE/d.

DISCUSSION

The total O_2 consumption of the duodenal mucosa of the sheep was higher than that calculated for the entire GI tract of fed adult sheep (4·65 nmol O_2/mg per min; Webster & White, 1973). This would be expected as the mucosal biopsies were devoid of serosal muscle layers. In comparison with the uptake of 6·55–7·18 nmol O_2/mg per min by the small intestinal mucosa of rats (Levin & Syme, 1975; Liberman *et al.* 1979), the O_2 consumption of sheep intestinal mucosa was lower. This result is consistent with the higher maintenance energy expenditure per unit body-weight of rats (750 ml O_2/kg body-weight per h; Levin & Syme, 1975) compared with sheep (328 ml O_2/kg body-weight per h).

In mucosal biopsies incubated in buffers containing either acetate or glucose, the Na^+,K^+-ATPase-dependent respiration rates were identical and accounted for approximately 50% of total O_2 uptake. Thus, Na^+,K^+-ATPase activity required to support the active uptake of glucose is a minor component of total Na^+,K^+-ATPase activity of the duodenal mucosa of mature sheep. This is supported by work reported by Scharrer (1975), which showed that active uptake of glucose does not take place in the small intestinal mucosa of mature ruminants. In the rat, however, 10–15% of Na^+,K^+-ATPase activity of the intestinal mucosa may be linked to the active uptake of glucose (Quigley & Gotterer, 1969).

Na^+,K^+-ATPase-dependent respiration accounted for approximately 50% of the total O_2 consumption of the duodenal mucosa of sheep given 7·6 mJ DE/d. This result was repeatable in the same animals, as evidenced by similar values obtained 3 months apart. Similarly, the estimation of Na^+,K^+-ATPase-dependent respiration was duplicated in mucosal biopsies incubated in Na^+-free media. The agreement between estimates of Na^+,K^+-ATPase-dependent respiration in mucosal biopsies derived from using ouabain, which is a specific inhibitor of Na^+,K^+-ATPase, and by incubation in a Na^+-free medium suggest that these estimates are indeed valid measures of Na^+,K^+-ATPase-dependent respiration and would not arise from altered intracellular concentrations of Na^+ and K^+ as suggested by Himms-Hagan (1976). Previous estimates of Na^+,K^+-ATPase-dependent respiration in the intestinal mucosa of ruminant species have not been reported in the literature, although Na^+,K^+-ATPase-dependent respiration may account for 35% of the total O_2 uptake of intestinal mucosa from fed rats (Levin & Syme, 1975; Liberman *et al.* 1979). Disparity between this estimate and ours may reflect species differences since higher proportions of Na^+,K^+-ATPase-dependent respiration have been found for the skeletal muscle of ruminants (35–40%; Gregg & Milligan, 1982*a*, *b*, *c*) compared with similar estimates for skeletal muscle of mice (14–22%; Gregg & Milligan, 1980 *a*, *b*).

Uptake of $^{86}Rb^+$ was used as a measure of Na^+,K^+-ATPase activity since rubidium is transported in the same manner as potassium (Love & Burch, 1953; Vaughan & Cook, 1972). The pattern of ouabain inhibition of $^{86}Rb^+$ uptake (Fig. 2) was similar to results derived from polarographic measurements made for Na^+,K^+-ATPase-dependent respiration. Ouabain caused an immediate 50% reduction in both O_2 and $^{86}Rb^+$ uptake of the duodenal

mucosa of sheep given 7·6 MJ DE/d. The extent of inhibition of both O_2 and $^{86}Rb^+$ uptake also remained constant for the duration (60 min) of the measurements. The agreement between these two measures of Na^+,K^+-ATPase activity suggests that ouabain is indeed a specific inhibitor of Na^+,K^+-ATPase and supports the use of ouabain-inhibited O_2 uptake of tissues as a direct and accurate method to measure the energy expenditure associated with ion transport.

The abdominal organs may account for up to 34–40% of the total heat production of mature animals (Webster, 1981). The GI tract contributes substantially to this total. In sheep, the GI tract may account for 10% of the total heat production in fasted animals (Edelstone & Holzman, 1981) and up to 15% of the total in fed animals (Webster, 1980). This estimate seems to vary in proportion to feed intake. The increase in heat production exhibited by animals after feeding has long been termed the heat increment of feeding (Kellner, 1926). The heat production from the GI tract, shortly after feeding, accounts for a significant proportion of the heat increment of feeding (Webster, 1980). This increase in heat production in the gut wall following feeding has been generally accepted to be the result of an increase in metabolic processes associated with digestion. Webster (1980) also suggested that elevated metabolic rates exhibited by gut mucosa of animals receiving high intakes may be related to higher protein synthesis rates in these tissues. Our results suggest that the maintenance of Na^+ and K^+ gradients across the plasma membrane of mucosal cells is also an energetically costly cellular function.

Weekes (1973), as cited by Webster (1980), concluded that the total metabolic activity of rumen epithelium remained relatively constant with increasing food intake. Similar tendencies were observed for duodenal mucosa in the present study. However, the present study indicates that there is likely to be a shift in the components of energy expenditure by the mucosal cells, as evidenced by the increase in Na^+,K^+-ATPase-dependent respiration per unit weight of duodenal mucosa.

It is of interest to note that Na^+,K^+-ATPase-independent respiration was higher in the intestinal mucosa of starved sheep compared with fed sheep. Na^+,K^+-ATPase-independent respiration would include the total O_2 uptake required for oxidative phosphorylation to support all of the endergonic cellular processes (e.g. protein synthesis, nucleic acid synthesis, Ca^{2+} transport) other than Na^+ and K^+ transport. These components of cellular energy expenditure were not measured, therefore the causal factors yielding the observed changes in Na^+,K^+-ATPase-independent respiration of ovine intestinal mucosa cannot now be quantitatively identified. Similarly, the relations between the Na^+-pump and these other cellular functions remain to be examined in future studies.

The results of the present study show that the magnitude of Na^+,K^+-ATPase-dependent respiration of duodenal mucosa can change with the level of DE intake. Previous work from our laboratory indicates that an increase in feed intake does not change tissue sensitivity to ouabain. The concentration of ouabain required for one-half maximal inhibition of Na^+,K^+-ATPase-dependent respiration in skeletal muscle of sheep did not differ between groups given either 950 or 1450 g lucerne hay/d (Gregg & Milligan, 1982*a*). Therefore, the increase in Na^+,K^+-ATPase-dependent respiration of duodenal mucosa with increasing intakes in the present study appears to be a real increase in Na^+,K^+-ATPase activity within the tissue. Similarly, Flier *et al.* (1981) found that when the energy intake of mice was increased by 30% above control levels, the Na^+,K^+-ATPase activity in their livers and skeletal muscles rose by 26 and 88% respectively. The mechanism by which either an increase in the number of Na^+,K^+-ATPase units or an activation of existing enzyme in duodenal mucosa of sheep is achieved in response to increased intake is an obvious topic for further investigation.

In conclusion, Na^+,K^+-ATPase-dependent respiration accounts for 28–60% of in vitro

duodenal mucosa O_2 consumption. Furthermore, the exact magnitude of this component of energy expenditure is influenced by the energy intake of the sheep, being greater at high levels of DE intake.

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EXPLANATION OF PLATE

Plate 1. Morphology of the duodenal mucosal biopsies from sheep given 7·6 MJ digestible energy/d. (*a*) Cross-section through a mucosal biopsy. The section, stained with haematoxylin and eosin shows the uniform development of intestinal villi. (*b*) Scanning electron micrograph of a villus of the duodenal mucosa. The uniform conical structure is evident and the biopsy is devoid of bacterial contamination. (*c*) Scanning electron micrograph of the epithelial cells covering the surface of a villus from a duodenal mucosa biopsy. (→) Extensive microvilli extensions of the epithelial cells.

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