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THE UNIVERSITY OF ALBERTA

ENERGY EXPENDITURE ASSOCIATED WITH NA+ AND K+ TRANSPORT IN HEPATOCYTES FROM SHEEP AND DUODENAL MUCOSA FROM CATTLE AND SHEEP

by

(C) Brian William Thomas McBride

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

[illegible] FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

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March 7, 1984

Dr. L. P. Milligan
Department of Animal Science
University of Alberta
Edmonton, Alberta
T6G 2P5

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The copyright laws are currently being revised; I believe the revisions are largely intended to strengthen the author's position further. In any case, they should not come into effect for several months.

I have enclosed photocopies from the Revised Statutes of Canada, 1970, which [illegible]

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To Whom It May Concern

As Mr. McBride's advisor (the member of academic staff that he studied with) for his Ph.D. program, it is entirely logical and traditional that I am a co-author of scientific publications arising from the thesis work. Of course, a primary purpose of this work is for him to satisfy the requirements for the Ph.D. degree and, therefore, there is absolutely no question that I, as a co-author of the articles

1) Development of a technique for gastrointestinal endoscopy of domestic ruminants. Can. J. Anim. Sci. 63: 349-354 (1983).

2) Endoscopic observations of the reticulo-omasal orifice of cattle. J. Agric. Sci., Camb. (1983) 101: 749-750.

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Œuvre exécutée dans l'exercice d'un emploi

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...itation ...re author is ...t owner of ...yright

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...nership in ...e of par...

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Supervisor

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....

External Examiner

Abstract

The purpose of this study was to define the energy cost of Na^+/K^+-transport, as measured by ouabain-sensitive respiration, in the small intestine and liver of growing, lactating or starved animals. The O_2 uptakes and ouabain-sensitive respiration rates of mucosal and liver biopsies, and hepatocytes were measured polarographically in an O_2 electrode assembly. Energy expenditure in support of Na^+, K^+-ATPase accounted for 55% of total duodenal mucosa respiration of cows at peak lactation. In late lactation and during the non-lactating period, the proportion of O_2 uptake inhibited by ouabain declined ($P<0.05$) to 34 - 35%. The amount of ouabain-sensitive respiration also declined from 2.14-2.39 to 1.47-1.66 nmol O_2/mg/min, during these periods.

O_2 consumption and Na^+, K^+-ATPase-dependent and -independent respiration of duodenal mucosa biopsies were measured for sheep fed two levels of digestible energy (DE) intake (7.6 and 14.8 MJ alfalfa/d) and following 48 h of starvation. Na^+, K^+-ATPase dependent respiration accounted for a significant proportion ([illegible]) of the total O_2 consumption of mucosal biopsies. The magnitude of this response was increased ([illegible]) to 61.3% of total mucosal O_2 consumption at the higher DE intake and was reduced ($P<0.01$) to [illegible] of total mucosal O_2 consumption during starvation. [illegible]

An in situ liver perfusion technique was developed for the isolation of ovine hepatocytes. Viability measurements, as assessed by trypan blue uptake, of 89.5 to 92.1% were achieved for isolated hepatocytes stored for up to 3 h on ice. Surface morphology of hepatocytes, as evaluated by scanning electron microscopy, did not change during 3 h of storage on ice. In lamb hepatocyte preparations with viabilities greater than 90%, ouabain-sensitive respiration accounted for 52.4 – 55.3% of total cellular O_2 consumption. Lamb hepatocyte preparations with viability of less than 50% exhibited lower ($P<0.05$) total and ouabain-sensitive respiration. The decrease in ouabain sensitive respiration in these preparations entirely accounted for the drop in total hepatocyte respiration.

O_2 consumption and ouabain-sensitive respiration was measured from liver biopsies of lactating and non-lactating ewes and from hepatocytes isolated from mature, dry ewes. O_2 consumption, ouabain sensitive respiration, $^{86}Rb^{+}$ uptake and ^{3}H ouabain binding were also measured from hepatocytes isolated from lambs, fed adult sheep and starved adult sheep. Ouabain sensitive respiration of liver biopsies from ewes in peak lactation accounted for 4[illegible]% of the total biopsy O_2 consumption. This proportion was 24 – 37% higher ($P<0.0$[illegible]) than similar measurements made during late lactation and during the non-lactating period. Total ouabain binding to hepatocytes was greater ($P<0.07$) for cells isolated from [illegible] sheep. The ext[illegible] binding to hepatocyte P<0.(?) for fed adult

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Acknowledgements

I thank Dr. R.T. Berg, past chairman, and Dr. R. Hardin, present chairman of the Department of Animal Science, for [illegible] of the departmental facilities.

I am deeply grateful to my mentor, Dr. [illegible] Milligan, for his continued support, time and advice given throughout this project. I have learnt greatly from his teachings and commitment to striving for academic excellence. I would also like to thank Drs. J.R. Thompson and R.J. Christopher [illegible] this project.

The [illegible] help and friendship given to me by the staff members of the Metabolic Unit, [illegible] Francis, C. Shellian [illegible] Gregory [illegible] is greatly appreciated. Special gratitude is [illegible] extended to my friend and [illegible] Dr. P. [illegible], for [illegible] uncompromising assistance throughout this project. The [illegible] and friendship [illegible] appreciated.

[illegible]

[illegible] the Na [illegible]

I [illegible] to dedicate this thesis [illegible] Her [illegible]

[illegible] ad [illegible] en [illegible] [illegible]

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I. Introduction

It is now realized that the maintenance energy expenditure of animals is not constant but can vary depending upon the animal's age and physiological state (Moe, 1981; Garrett and Johnson, 1982). However, the metabolic components comprising maintenance energy expenditures are only now being defined quantitatively. One major component of maintenance energy expenditure is Na^+/K^+-transport (Milligan, 1971). The transport of Na^+ and K^+ across the plasma membrane of animal cells is controlled by Na^+, K^+-ATPase. The extrusion of 3 Na^+ out of the cell and concerted uptake of 2 K^+ into the cell is accomplished with the expenditure of 1 ATP (Mandel and Balaban, 1981). Therefore, any physiological changes in the animal's metabolism that influences the rate of Na^+/K^+-transport will directly affect the maintenance energy expenditure of animal tissues, especially considering that the support of Na^+, K^+-ATPase may account for 20–[illegible]0% of the total energy expenditure of many animal tissues (Ismail-Beigi and Edelman, 1970; 1971; Balaban et al., 1980).

The purpose of this study was to determine: (1) the magnitude of energy expended in support of Na^+/K^+-transport in intestinal mucosa and liver of sheep and cattle and (2) the influence of the animal's physiological state on the magnitude of this maintenance energy expenditure. Intestine and liver were examined because past research has shown that Na^+, K^+-ATPase activity in intestinal mucosa and liver may [illegible] one-third of the total energy expenditure of these tissues in rats (Ismail-Beigi and Edelman, 1970; 1971; Liberman et al. 1979). Furthermore, these organs may account for up to 10 - 20% of the total energy expenditure of animals (Webster, 1980; 1981; Edelstone and Holzman, 1981).

A site specific endoscopy procedure[1] was developed to allow for repeatable sampling of the intestinal mucosa of sheep and cattle. An in situ liver perfusion technique was also developed to isolate ovine hepatocytes. These methods provided viable tissues or cells which reflected the physiological status of the sampled animal. Energy expenditure associated with Na^+/K^+-transport of intestinal mucosa and hepatocytes was assessed by ouabain sensitive respiration using established in vitro procedures.

---

[1]The endoscopy procedure and further ar[illegible] of the [illegible]

II. The Effect of Lactation on Ouabain-Sensitive Respiration of the Duodenal Mucosa of Cows

A. Introduction

Maintenance energy expenditure is a component of [illegible] energy expenditure of young growing animals and mature animals in a state of energetic equilibrium. However, the magnitude and metabolic components of maintenance energy expenditures of animals appear to change in response to changes in physiological state. Webster (1978) indicated that growing animals have a higher maintenance energy component of total energy expenditure than do mature animals. Similarly, lactating cows have a higher maintenance energy expenditure than non-lactating cows (Moe, 1981). Changes in the composition of tissue deposition during these different physiological states may account for some of these differences in maintenance energy expenditure (Lister, [illegible]; Webster, 1981) but the question of what quantitatively comprises the metabolic components of maintenance [illegible] remains. It has been suggested that the maintenance of [illegible] gradients across the plasma membrane [illegible] K ATPase may [illegible] cellular maintenance costs (Milligan, 19[illegible]). This suggestion has been confirmed by [illegible] workers for a number of different tissues. [illegible] workers have found that the [illegible] ATPase activity in tissue [illegible]

30-40% of the total energy expenditure in these tissues (Ismail-Beigi and Edelman, 1971; Asano et al. 1976; Lo et al. 1976). These measurements have also been confirmed recently for liver and kidney by other research groups (Mandel and Balaban, 1981; Van Dyke et al. 1983). However, most of these estimates of Na , K⁺-ATPase-dependent respiration were made for tissues of mature adult [illegible]ats, [illegible] fed at maintenance. Work from our laboratory has shown [illegible] Na , [illegible]-ATPase dependent respiration may also account for [illegible] skeletal muscle respiration in [illegible] stressed [illegible] and growing lambs and cal[illegible] ([illegible] and Milligan, 198[illegible] a,b,c . Therefore, the [illegible] [illegible] study was to determine the magnitude of Na , [illegible]-ATPase-dependent respiration (or ouabain-sensitive respiration) of duodenal mucosa in cows imposed with the physiological stress of [illegible]. Tissues of the gut were [illegible] for study [illegible] significance [illegible] [illegible]

[illegible]

[illegible]

Two [illegible] Holstein [illegible] duodenal cannulae [illegible]. The animals were fed tw[illegible] [illegible] tra[illegible]

[illegible]

rapeseed meal, 1.2% calcium phosphate, [illegible] 4% calcium carbonate, [illegible] 3% trace mineralized salt and 0.[illegible] vitam[illegible] ADE) and chopped alfalfa hay according to their milk production. The feed int[illegible] during the [illegible]th, 16th, 19th, [illegible]

[illegible]

assembly. Air saturated KH buffers (37°C, 700 mm Hg, 180 μmol O_2 ml; Umbreit et al. 1964) were used in all O_2 consumption measurements. Initial O_2 consumption was measured for 15 min and the biopsies were transferred to another electrode chamber containing 4 ml of KH buffer, having ouabain concentration of 0, 10 [illegible], 10 [illegible], 10^{-5} or 10 [illegible] M. O_2 uptakes of these biopsies were measured for a further 40-45 min. The difference between the initial O_2 consumption and the O_2 consumption of the ouabain treated samples was termed ouabain sensitive respiration. Mean percent inhibition of respiration by ouabain was determined in duplicate for each ouabain concentration and a dose response curve was constructed expressing inhibition as a percentage of maximum inhibition. Based upon the dose response, subsequent measurements of ouabain sensitive respiration were conducted in the KH buffer containing [illegible]

Histology of the Duodenal Mucosa

Mucosal biopsies were excised from the descending duodenum of the cows, then were fixed, stained and sectioned according to the method of [illegible] et al. (19[illegible]). The [illegible] sections [illegible] to determine if the [illegible] epithelium [illegible] mucosal biopsies were also prepared to determine the extent of colonization of gut bacteria on the epithelium and to examine the surface morphology of the intestinal epithelium. The biopsies were mounted on nylon mesh (Perera et al., 19[illegible]5) and then were washed with ice cold phosphate buffer (63.2 [illegible] $Na_2HPO_4 \cdot 7H_2O$; 15.0 mM $NaH_2PO_4 \cdot H_2O$; pH 7.4) and fixed using phosphate buffer as the solvent. The samples were fixed [illegible] 1% glutaraldehyde for 24 h at 4°C then were post fixed in 1% OsO_4 for 2 h at 4°C. Following double fixation, the biopsies were dehydrated in a graded ethanol series and were taken to 100% amyl acetate in a stepwise fashion. From 100% amyl acetate, the samples were critical point dried in CO_2 (Anderson, 1951) then were coated with gold palladium. Finally, the prepared biopsies [illegible] [illegible] [illegible] [illegible] [illegible] [illegible]

[illegible]

Statistical Analysis

All data [illegible] [illegible] [illegible] [illegible]

[illegible]

Morphology of the Duodenal Mucosa

Histological examination of the duodenal mucosa of the cows indicated that the biopsies were free of the longitudinal and transverse muscle layers (Plate II.1a). Hypertrophy of the mucosa appeared evident throughout lactation. This was evidenced by enlarged Brunner's glands, fused and truncated villi and increased depth of villus crypts (Plate II.1a; Fell et al. 1964). These changes resulted in a thickened intestinal mucosa. Our findings support previous reports that have shown hypertrophy of the gastrointestinal tract, particularly the small intestine, during lactation (Fell et al. 1964; Fell et al. 1972).

The scanning electron micrographs of the mucosa clearly showed the prominence of the intestinal villi (Plate II.1b) and the dense covering of microvilli on the surface of the epithelial cells (Plate II.1c). The epithelial cells appear as polygonal structures along the surface of villi (Plate II.1[illegible]). At higher magnifications, the microvilli appear as [illegible] covering of the epithelial cells. The microvilli were uniform in both length and width (Plate II.[illegible]). Furthermore, in all biopsies examined under the scanning [illegible] the surface of the epithelium [illegible] [illegible] the [illegible] of the [illegible] [illegible] [illegible]

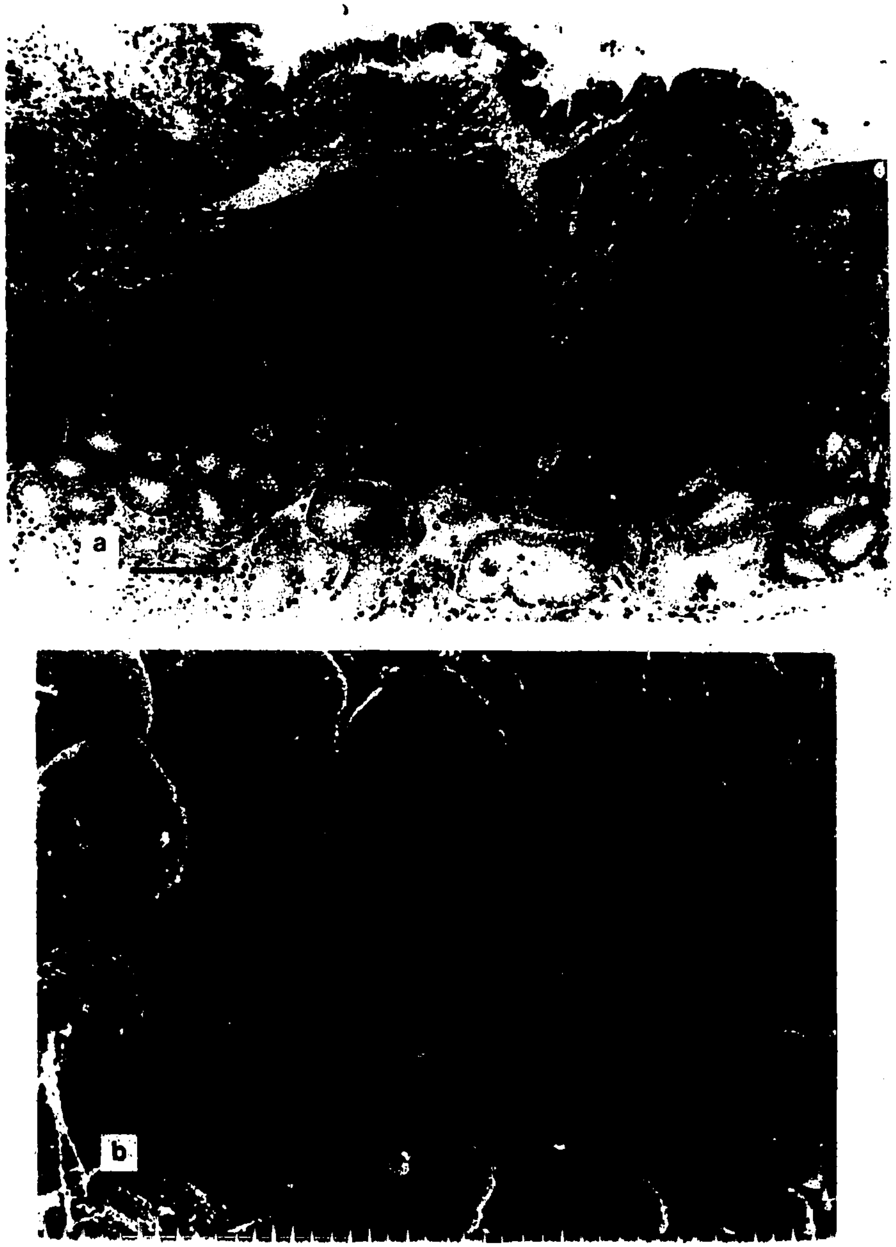


Plate II.1a,b. Morphology of duodenal biopsies. a) Cross-section of a duodenal biopsy from a Holstein cow in the 2[illegible]th wk of lactation (H and E stain; Bar-0.1 mm). The upper arrow points to a broad truncated villus. The lo[illegible] arrow indicates the extensive development of Brunner's glands [illegible], and [illegible] graph [illegible] duode[illegible]
[illegible]

Plate II.1c. Scanning electron micrograph of a duodenal villus of a Holstein cow in the 19th wk of lactation. The [illegible] points out the [illegible] surface of a columnar epithelial [illegible]

Inhibition of Mucosal O_2 Consumption By Ouabain

The response-dose curve of the duodenal mucosa to ouabain was sigmoidal in shape (Fig. II.1). The lowest concentration of ouabain that caused maximum inhibition was 10^{-5} M. This is a higher concentration than that needed for maximum inhibition of respiration of skeletal muscle from sheep and cattle (Gregg and Milligan, 1982 a,b) but lower than that used for inhibition of jejunal mucosa Na^+, K^+-ATPase of rats (10^{-3} M; Liberman et al. 1979). These differences in the estimates of a minimal ouabain concentration required to induce maximal inhibition of O_2 consumption may reflect differences in sensitivity of both species and tissue-type to ouabain (Tobin and Brody, 1972; Tobin et al. 1972). The ouabain-dose response curve was examined only at peak lactation, when ouabain-inhibitable O_2 uptake was at a maximum (Table II.1). We assumed that the concentration of ouabain yielding maximum inhibition of respiration at this period would also produce maximum inhibition at other stages of lactation.

The actual percentage of total Na^+, K^+-ATPase activity that was inhibited by ouabain was not measured, therefore estimates presented here likely constitute minimal measurements of Na^+, K^+-ATPase dependent respiration, since absolute cessation of Na^+, K^+-ATPase activity would require access of ouabain to all of the enzyme units in the tissue.

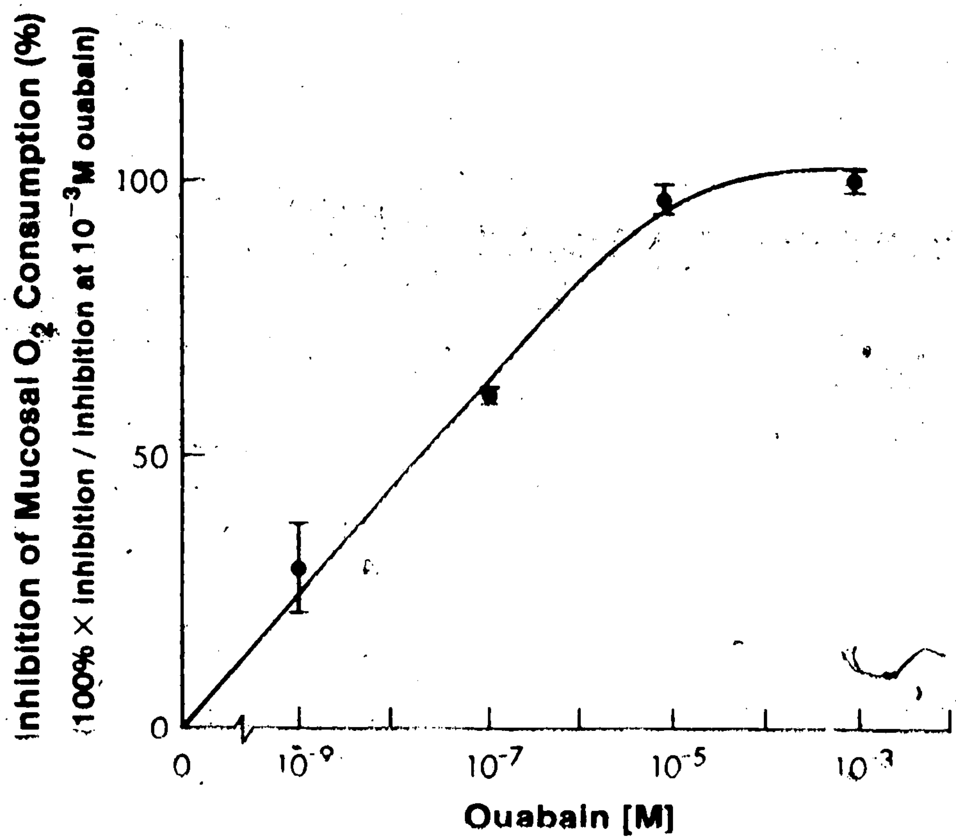


Figure II. 1.
Inhibition by ouabain of respiration of duodenal mucosa of a cow during mid-lactation. Inhibitions are expressed as a percentage of inhibition at 10^{-3}M ouabain. Means are given with standard errors.

Table II.1. Effect of lactation on O_2 consumption, percent inhibition of O_2 consumption by ouabain, ouabain-sensitive and ouabain-insensitive respiration in duodenal mucosa of cows. Results are expressed as means ± S.E.M.*

Physiological State	Milk Yield (kg/d)**	Total O_2 Consumption (nmol O_2/mg/min)	Percent Inhibition (%)	Ouabain-Sensitive Respiration (nmol O_2/mg/min)	Ouabain-Insensitive Respiration (nmol O_2/mg/min)
Lactating					
5 wk	25 ± 1 6*a*	3.89 ± 0.41*a*	53.8 ± 5.0*a*	2.14 ± 0.38*a*	1.75 ± 0.16*a*
16-19 wk	4.8 ± 0.8*b*	4.35 ± 0.41*a,b*	55.0 ± 2.6*a*	2.39 ± 0.26*a*	1.96 ± 0.20*a*
22-25 wk	0 1 ± 0 5*c*	4.92 ± 0.22*b*	33.7 ± 2.9*b*	1.66 ± 0.18*a,b*	3.26 ± 0.18*b*
Pregnant, non-lactating		4.19 ± 0.17*a*	34.9 ± 1.4*b*	1.47 ± 0.11*b*	2.72 ± 0.10*c*

a,b,c Means within columns followed by different letters are significantly ($P<0.05$) different.

* All respiration results are expressed on a mg dry weight basis.

** Means within this column followed by different letters are significantly ($P<0.10$) different.

O_2 Consumption and Ouabain-Sensitive and -Insensitive Respiration of Duodenal Mucosa

Total mucosal O_2 consumption increased ($P<0.05$) from early to mid-lactation and fell to initial O_2 uptakes during the dry period (Table II.1). The highest rate of mucosal O_2 consumption (4.92 nmol O_2/mg/min) was found during the 22-25 wk of lactation. This measurement also corresponded with the highest rate of ouabain-insensitive respiration recorded for duodenal mucosa during lactation and may reflect a consequence of gut hypertrophy during lactation.

The absolute values of total mucosal O_2 consumption rates of cows throughout lactation were similar to values reported by Webster and White (1973) for the entire gastrointestinal tract of fed sheep (4.65 nmol O_2/mg/min) and less than those reported for jejunal mucosa of adult rats (6.55-7.18 nmol O_2/mg/min; Levin and Syme, 1975; Liberman et al. 1979). The higher O_2 consumption reported for the mucosa of rats certainly is consistent with the higher maintenance expenditure, expressed per unit of metabolic weight, of these animals compared to cattle (Webster, 1981).

Support of Na^+, K^+ ATPase activity in duodenal mucosa of cows appears to be a major energy expenditure accounting for up to 55% of the total O_2 uptake of the duodenal mucosa of lactating cows (Table II.1). The magnitude of this component of respiration also appears related to the stage of lactation. At peak lactation, ouabain-sensitive respiration was 53.8-55.0% of the total mucosal O_2 consumption. During mid-lactation and the dry period, the magnitude of ouabain-sensitive respiration declined. In the non-lactating cow, ouabain-sensitive respiration dropped ($P<0.05$) to about two-thirds that at peak lactation (Table II.1). Similarly, the proportion of respiration sensitive to ouabain fell ($P<0.05$) from approximately 54% to 34.9% from peak lactation to the non-lactating period.

Ouabain-insensitive respiration of the mucosa also changed ($P<0.05$) with stage of lactation. The ouabain-insensitive component of total mucosal respiration was 86 and 55% greater during mid-lactation and the dry period than during early lactation (Table II.1). O_2 uptake to support the energy cost of cellular syntheses would be included in the ouabain-insensitive component of respiration. There would be a high rate of energy expenditure required to support the rapid protein synthesis that occurs in the gastrointestinal tract (Davis et al 1980; McNurlan and Garlick, 1980) including that of lactating cows (Oldham et al. 1980).

The proportion total respiration that was ouabain-sensitive during the first half of lactation (53.8-55%) was higher than that found by Liberman et al.(1979) for jejunal mucosa for rats (35%). However, Liberman and coworkers used adult rats fed at maintenance in their study. Higher levels of ouabain sensitive respiration in mucosa might be expected from rats under [illegible] demanding physiological states. It is of interest to note that the proportion of ouabain-sensitive respiration in the duodenal mucosa of non-lactating cows, fed at maintenance, was similar to those reported for adult, maintenance-fed rats (30-35%; Levin and Syme, 1975; Liberman et al. 1979).

Clearly, the energy expended in the support of Na^+ and K^+ gradients across the plasma membrane is a major and variable component of maintenance energy expenditure of the intestinal mucosa of lactating and dry pregnant cows. Moe (1981) observed that the partial nutritional efficiency (see Milligan, 1971) for milk production by cows was 64%, when the maintenance energy cost of the animal was assigned a constant value of 510 kJ ME/$kg^{0.75}$. Our results indicate that a single estimate for maintenance energy expenditure throughout lactation may oversimplify the true physiological expression of maintenance certainly in relation to the activity of the intestinal mucosa. Considering that maintenance of Na^+, K^+-ATPase accounts for 33.9% to 55% of the total mucosal O_2 consumption and the magnitude of this response changes in relation to the stage of lactation, it is reasonable to suggest that very different maintenance energy costs may also be apparent throughout lactation in other tissues of the body. Previous work from our laboratory has shown that the energy expenditure to support ion transport also increases in the skeletal muscle of ewes during lactation (Gregg and Milligan, 1982 c). Therefore, maintenance expenditure may not be a constant component of total energy expenditure throughout lactation

III. Influence of Feed Intake and Starvation on the Magnitude of Na^{+}, K^{+}-ATPase Dependent Respiration in Duodenal Mucosa of Sheep

A. Introduction

Na^{+}, K^{+}-ATPase (EC 3.6.1.3)-dependent respiration has been found to be a major component of cellular energy expenditure in tissues such as skeletal muscle, brain and abdominal organs such as liver, intestine and kidney (Ismail-Beigi and Edelman, 1971; Asano et al. 197[illegible]; Liberman et al. [illegible]). The magnitude of the Na^{+}, K^{+}-ATPase response appears to be related to the function of the tissue and physiological status of the animal (Ismail-Beigi and Edelman, [illegible]; [illegible] and Balaban, 1981). In mammalian kidney Na^{+}, K^{+}-ATPase [illegible] Na^{+} reabsorption in the kidney accounts for [illegible] 0% of the total kidney [illegible] uptake (Balaban et al. 1980). [illegible] liver Na^{+}, K^{+}-ATPase activity is not [illegible] and therefore [illegible] of the tissue [illegible] 1981) [illegible] of Na^{+} [illegible] dependent respiration in tissues also [illegible] physiological [illegible] kidney [illegible] exhibiting higher metabolic rates due to the physiological stresses of cold (Gregg and Milligan, 1982a) and lactation (Gregg and Milligan, 1982)

The gastrointestinal tract (GI) has been proposed as major site of [illegible] (1981) a [illegible] K [illegible] 1979) [illegible] the [illegible]

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 2h following morning feeding on two consecutive days during each period of the energy intake regimes. For the starved animals, two biopsies were taken from each animal [illegible] consumption [illegible]

[illegible] Animal Respiration

[illegible] animal [illegible] rates were determined for each [illegible] intake regimes and following the 48h [illegible]

[illegible] of [illegible] to Ouabain

[illegible] single sheep fed the lower [illegible] Krebs Henseleit buffer [illegible] D-glucose [illegible] mm Hg, [illegible] pH 7.4 [illegible] concentrations of 0, 10 [illegible], 10 [illegible], 10 [illegible] or 10 [illegible] M. O_2 uptakes of these biopsies were measured for a further 40 [illegible]5 min during which time the O_2 uptakes remain[illegible] line[illegible]. The reduction in the rate of O_2 consumption in the ouabain-treated samples w[illegible] ter[illegible] Na^+, K^+ ATPase dependent respiration. Mean perce[illegible] inhibition of O_2 uptake b[illegible] oua[illegible] was calculated for each ouabain con[illegible]ntration [illegible] a [illegible] response was [illegible]onstructed expressing [illegible] [illegible]ntage of maxim[illegible]m inhibiti[illegible]

All subsequ[illegible]nt r[illegible]spiration m[illegible]asurements from the [illegible] biopsies were cond[illegible]ted using the methods descri[illegible] with exceptio[illegible] that Na[illegible] K[illegible] ATPase [illegible] dep[illegible] respira[illegible] determin[illegible]d from samples exposed [illegible] 2.[illegible] 10 [illegible] ouabain. The r[illegible]spirat[illegible] [illegible] expressed on a biopsy dry weight [illegible] [illegible] [illegible] measurements [illegible] [illegible]

[illegible] uptake [illegible]

[illegible] s[illegible]dium phosphate, 0.8[illegible] segment [illegible] [illegible]ped of the [illegible] of the Krebs-Henseleit buffer (pH 7.4, 37°C)containing 2% bovine serum albumin, 5uCi $^{86}Rb^+$, 0.1mM RbCl and 10mM D-glucose. Inhibition of $^{86}Rb^+$ uptake by ouabain over 10 min of incubation (37°C), was determined in triplicate for each concentration of ouabain. A 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^{-3} M ouabain on $^{86}Rb^+$ uptake by the mucosa biopsies was determined in triplicate for incubation periods of 1,5, 15 and 60 min.

Upon completion of the various incubations, ^{86}Rb uptake was stopped by aspiration of the medium, the mucosal samples were rinsed with ice cold phosphate buffered saline. The mucosal samples were transferred to 15ml plastic scintillation vials and 1 ml of Protosol (New England Nucleur, Boston, Mass.) was added to the samples. The samples were subsequently digested for 1h at 55°C in a shaking water bath. Glacial acetic acid (50ul) was added to neutralize the dissolved samples; 10 ml of Unisolve 1 (Terochem Laboratories Ltd., Edmonton, Alb.) 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 are expressed as dpm/mg dry weight. The wet weight of each sample was converted to a dry weight basis [illegible] the dry matter percentage of 14.5 ± 1.5% determined [illegible] from the same animal

Morphology of the Duodenal Mucosa 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 regimes. The samples were preserved, dehydrated, sectioned and stained according to the procedures of Perera et al. (1975).

Scanning electron micrographs were taken of the mucosal biopsies to assess the surface morphology of the biopsies and to determine the extent of gut bacteria colonization on the duodenal epithelium. The excised biopsies were washed with phosphate buffer (63.2 mM $Na_2HPO_4 \cdot 7H_2O$; 15.0mM $NaH_2PO_4 \cdot H_2O$; pH 7.4) and were fixed with phosphate buffer solvents.

The samples were mounted on nylon mesh (Perera et al. 1975) fixed in 1% glutaraldehyde for 24h at 4°C then post fixed in 1% OsO_4 for 2h. The samples were subsequently dehydrated in a graded ethanol series and were taken to 100% amyl acetate before critical point drying in CO_2 (Anderson 1951). The biopsies were coated with gold palladium then were scanned on a Cambridge Ste[illegible]

[illegible]

Analysis of Results

Results are expressed as means followed by their standard errors. All data were analyzed by ana[illegible]

[illegible]

t-tests or by Student-Newman-Keul's multiple range tests (Steel and Torrie, 1960).

C. Results

Morphology of the Mucosal Biopsies

The histological and fine structure of the duodenal mucosal biopsies are shown in Plates III.1a, b and c. The biopsy procedure yielded an intact mucosal preparation devoid of the serosa and the longitudnal and transverse layers of the muscularis externa (Plate III.1a). The structural integrity of the duodenal mucosa and the abundance of the intestinal villi did not appear to change at the different energy intake regimes. Plates III.1b 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 III.1b). 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 III.1c). Similar morphological features were present in mucosal biopsies [illegible]

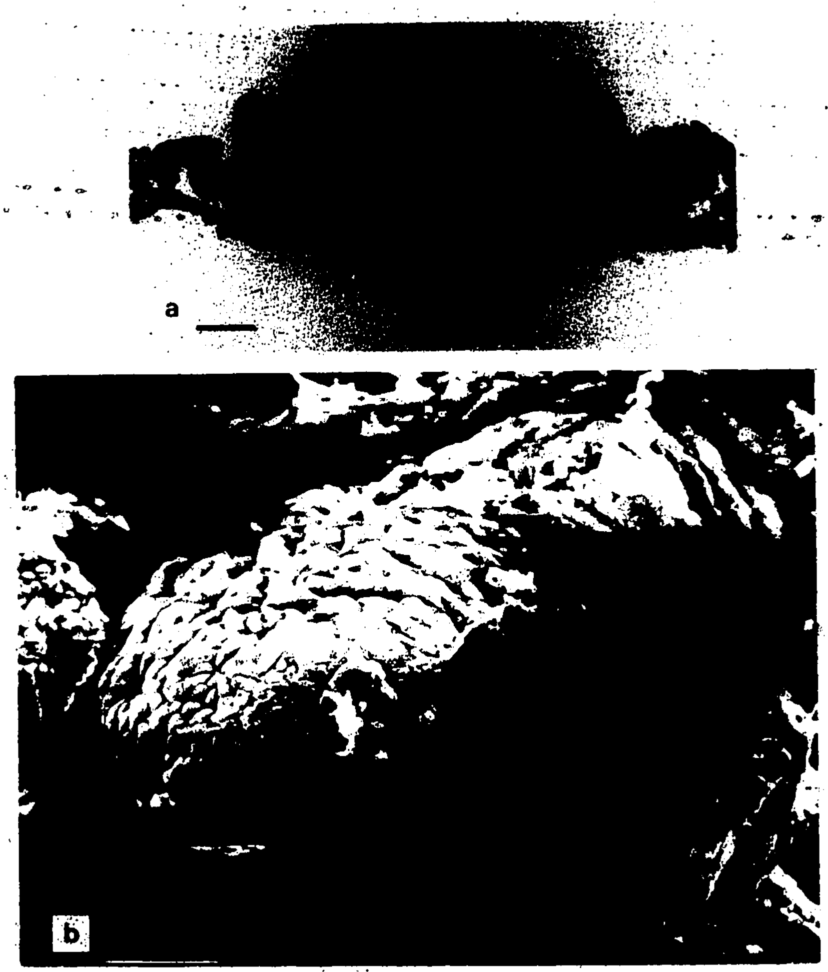


Plate III.1a,b. Morphology of the duodenal mucosal biopsies of sheep fed 7.6 MJ DE/d. (a) Cross-section through a mucosal biopsy. The section shows the uniform development [illegible] intestinal villi. The section was stained with H & E. Bar= 0.25 mm. (b) A scanning electron micrograph of a villus of the duodenal mucosa. The uniform conical structure of the villus is evident. [illegible]he biopsy is [illegible] [illegible] [illegible]

Plate III.1c. A scanning electron micrograph of the epithelial cells covering the surface of a villus of a duodenal mucosa biopsy. The extensive microvilli extensions of the epithelial cells is pointed out with the arrow. Bar= 1 μm.

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. III.1. The shapes of the ouabain-dose-response curves were sigmoidal for both measurements. The lowest concentrations of ouabain yielding maximum inhibition were 10 [illegible] and 10^{-4}M, for the O_2 and $^{86}Rb^+$ uptake measurements, respectively.

The time course of ouabain inhibition of $^{86}Rb^+$ uptake is shown in Fig. III.2. Maximum inhibition of $^{86}Rb^+$ uptake by the mucosal biopsies was reached within 5min 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.

O_2 Uptake and Na^+, K^+-ATPase-Dependent Respiration

Total O_2 uptake and Na^+, K^+-ATPase-dependent respiration of duodenal mucosa are shown in Tables III.1 and III.2. The use of acetate as a substrate instead of gluc[illegible] caused an insignificant ($P>0.05$) reduction in total O_2 consumption of duodenal mucosa (Table III.1). This slight drop in O_2 consumption was entirely accounted for by a [illegible] ($P>0.05$) decrease in the Na^+, K^+-ATPase independent respiration; extent of ouabain inhibition and Na^+, K^+-ATPase dependent respiration were not influenced ([illegible]) by the [illegible]

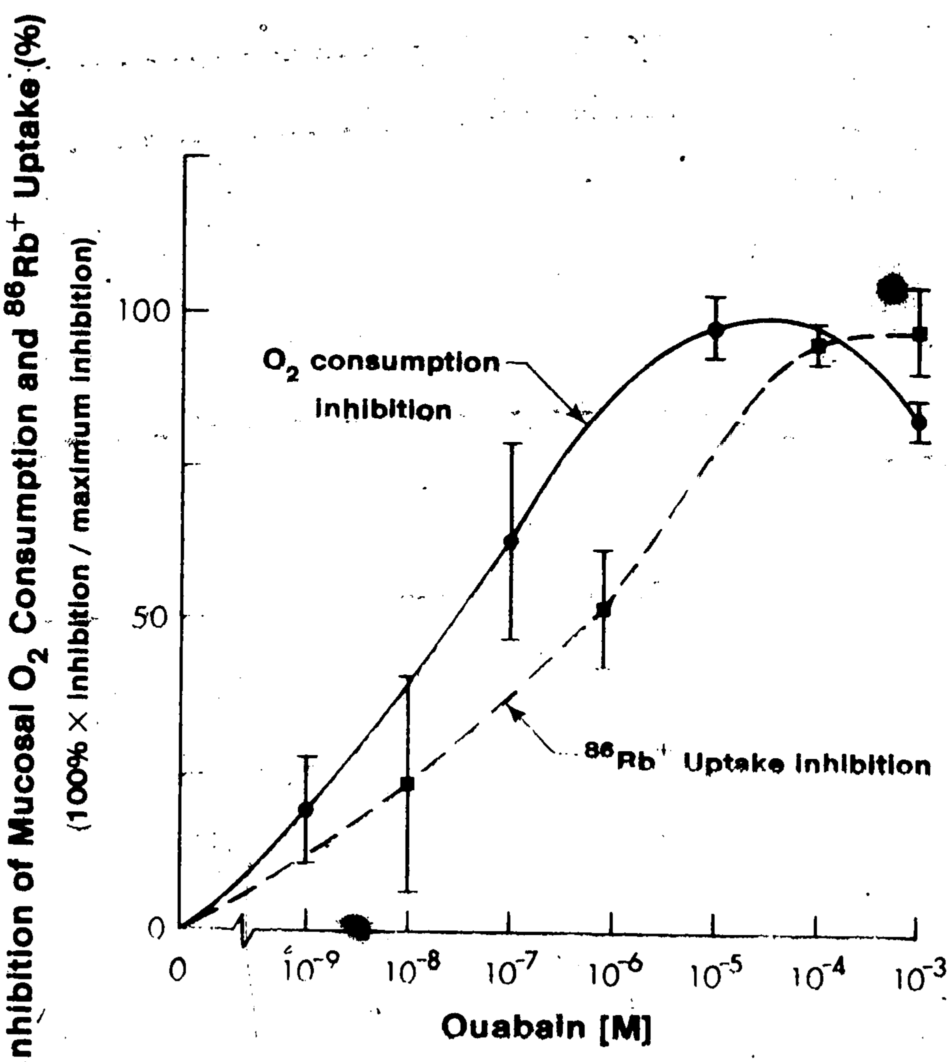


Figure III. 1.
Inhibition by ouabain of respiration and $^{86}Rb^+$ uptake of duodenal mucosa of sheep fed 7.6 MJ DE/d. Inhibitions are expressed as a percentage of maximum inhibition. Values are means ± S.E.

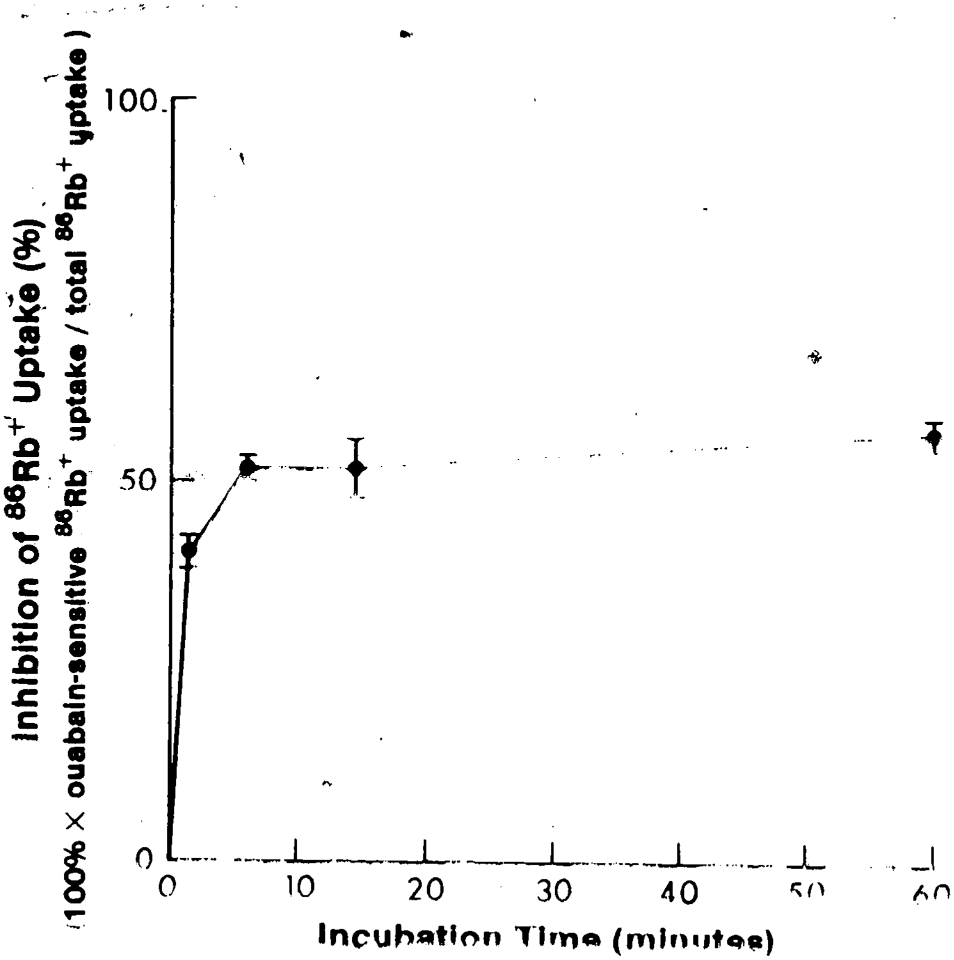


Figure III.2.
Time scale of ouabain inhibition of $^{86}Rb^+$ uptake. Inhibitions are ex-
pressed as ouabain-sensitive [illegible]
[illegible]

ble III.: otal O_2 consumption, percentage inhibition and Na^+, K^+-ATPase-dependent and -independent respiration of uodenal mucosa of sheep fed 7.6 MJ DE/d.* (Mean values with their standard errors).**

eatment	al consumption (mol O_2/mg/min) Mean	SE	Percentage inhibition of O_2 consumption Mean	SE	Na^+, K^+-ATPase dependent respiration (nmol O_2/mg/min) Mean	SE	Na^+, K^+-ATPase -independent respiration (nmol O_2/mg/min) Mean	SE
cetate	.32	0.28	50.1	.6	2.68	0.30	2.64	0.30
ucose	5	.41	48.1	.5	.69	0.20	2.96	0.33
a+-free	7	.43	45.5	2.8	.53	0.22	3.04	0.13
E2 glucose	54	.84	47.1	4.3	2.72	0.44	3.12	0.67

* Means within columns do not differ significantly ($P<0.05$).

** All respiration results are expressed on a mg dry weight basis.

Table II: Whole animal and mucosal O_2 consumption, percent inhibition and Na^+, K^+-ATPase-dependent and -independent respiration of duodenal mucosa of sheep fed two levels of digestible energy intake or starved for 48 h. (Means with their standard errors) **

ment	stible intake /d)		Whole animal consumption (kg/h)		Mucosal O_2 consumption (nmol O_2/mg/min)		Percent inhibition of O_2 consumption		Na^+, K^+-ATPase dependent respiration (nmol O_2/mg/min)		Na^+, K^+-ATPase -independent respiration (nmol O_2/mg/min)*	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ation		0a	36	[illegible]	21	23	3.6	[illegible] a	48	0.14a	3.73	0.25a
		[illegible]	28	[illegible]	55	11	[illegible]	5b	69	0.20b	2.96	0.33b
		3c	09	[illegible]	07	29	[illegible] 3	3c	59	0.25c	2.38	0.18b

Means within this column followed by different letters are significantly (P<0.05) different.

Means within a column followed by different letters are significantly (P<0.01) different.

Mucosal respiration rates are expressed on a mg dry weight basis

O_2 consumption, Na^+, K^+-ATPase-dependent respiration and Na^+, K^+-ATPase independent respiration and percent inhibition values measured for mucosal biopsies of sheep fed 7.6 MJ DE/d were similar when measured 3 months later after the animals had been returned to a similar DE intake of [illegible] MJ/d (Table III.1). Total O_2 consumption and Na^+, K^+-ATPase independent respiration were insignificantly increased in the mucosal biopsies upon re-measurement. Na^+, K^+-ATPase dependent respiration and percent inhibition of total O_2 consumption by ouabain were almost identical for mucosal biopsies between sampling periods.

The Na^+, K^+-ATPase dependent respiration of duodenal mucosa of sheep fed 7.6 MJ DE/d was also assessed [illegible] Na free medium (Table III.1). The estimate of Na^+, K^+-ATPase dependent respiration, derived from the use of Na free medium, was not different ($P>0.05$) from the estimate [illegible] for Na^+, K^+-ATPase dependent respiration using ouabain as an inhibitor of Na^+, K^+-ATPase. Furthermore, the total [illegible] consumption, Na^+, [illegible] respiration and percent inhibition [illegible] Na^+, K^+-ATPase [illegible] Na free medium [illegible]

[illegible]

[illegible] consumption of [illegible] fed 7.6 MJ DE/d was 37% higher (P [illegible]) than [illegible]. At the higher (11.8 MJ/d) DE intake, [illegible] rates of the [illegible] were 20% great[illegible] (P [illegible]) than at the lower (7.6 MJ/d) DE intake and [illegible] higher [illegible] compared to the [illegible] rates [illegible]

[illegible] differ [illegible] intake [illegible]

[illegible]

O_2 mg min; Webster and White, 1973). This would be expected as the mucosal biopsies were devoid of serosal muscle layers. In comparison with the O_2 uptake of the small intestinal mucosa of rats (6.55 7.18 nmol O_2 mg/min; Levin and Syme, 1975; Liberman et al. 1979), the O_2 consumption of sheep intestinal mucosa is lower. This result is consistent with the higher maintenance energy expenditure per unit body weight of rats ([illegible] ml O_2 kg BW h; Levin and Syme, 1975) [illegible] to sheep (328 ml O_2/kg BW h).

[illegible] mucosal biopsies incubated in buffers containing either acetate [illegible] glucose, the Na, [illegible] ATPase dependent respiration rates were identical and accounted for approximately [illegible] of total O_2 uptake. Na [illegible] ATPase dependent respiration represents the proportion [illegible] consumption required, in part at least, for the maintenance of Na and K gradients across [illegible] membrane. The results of this study show th[illegible] [illegible] required to support the active [illegible]

[illegible]

Na^+, K^+-ATPase-dependent respiration accounts for approximately 50% of the total O_2 consumption of the duodenal mucosa of sheep fed 7.6 MJ DE/d. This result was highly 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-Hagen (1976). Previous estimates of Na^+, K^+ ATPase dependent respiration in the intestinal mucosa of ruminant species have not been reported in the literature, although in the small intestinal mucosa of fed rats Na^+ [illegible] K^+ ATPase dependent respiration may account for [illegible] of the tissue [illegible] and Simon [illegible] [illegible]

Uptake of $^{86}Rb^+$ was used as a potassium tracer to measure Na^+, K^+-ATPase activity since rubidium is taken up in the same manner as potassium (Love and Birch, 1953; Vaughan and Cook, 1972). The pattern of ouabain inhibition of $^{86}Rb^+$ uptake (Fig. III.2) was similar to the 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 fed 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 inhibitable 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 gastrointestinal tract contributes substantially to the total. In sheep, the GI tract may account for 10% of the total heat production in fasted animals (Edelstone and Holzman, 1980) 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 i[illegible] of feeding ([illegible]). The heat producti[illegible] accounts for a significant proportion of the heat increment of feed (Webster, 1980). This increase in heat production in the gut wall following feeding has been generally accepted to result from 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 would suggest that the maintenance of Na^+ and K^+ gradients across the plasma membrane of mucosal cells is also an energetically costly cellular function. It might also be involved in the heat increment of feeding.

Na^+, K^+-ATPase-independent respiration, which represents the O_2 consumption associated with all other cellular processes, varied from 38.7-71.4% of the total mucosal O_2 consumption depending upon the animals' digestible energy intake. The exact components of this energy expenditure can only be speculated upon. Protein synthesis and Ca^{++} transport would undoubtedly contribute this proportion of cellular energy expenditure. However, the relationship between the Na pump and [illegible] functions remains to be discovered.

The results of this study show that the magnitude of Na^+, K^+-ATPase-dependent respiration of duodenal mucosa change with level of digestible energy intake and suggest [illegible] mucosal Na [illegible] reflect a mechanism to conserve energy during periods of depressed feed intake. Furthermore, the activity of Na^+, K^+-ATPase was rapidly modulated during this interval (48 h) of fasting. The decrease in activity could have resulted from either an actual or effective decrease in the number of enzyme sites or a decrease in activity of the existing enzyme units. Future studies, however, will have to be conducted to delimit the precise mechanism responsible for the regulation of Na^+, K^+-ATPase activity in the duodenal mucosa of sheep.

In conclusion, Na^+, K^+-ATPase-dependent respiration accounts for a significant proportion of in vitro duodenal mucosa O_2 consumption. Furthermore, the magnitude of this component of energy expenditure is influenced by the animals' energy intake being greater at higher levels of digestible energy intake.

IV. Magnitude of Ouabain-Sensitive Respiration of Lamb Hepatocytes

A. Introduction

A great deal of research in animal agriculture has been directed toward determining the components of whole body energy expenditure (Webster, 1981). To that end, work has been undertaken to ascertain the energy cost of cellular processes including protein synthesis (Reeds et al. 1982) and Na^+/K^+-transport, the latter being equated with ouabain-sensitive respiration (Gregg and Milligan, 1982 a,b,c). Protein synthesis accounts for a minimum of 20% of total daily energy expenditure or heat production of growing pigs (Reeds et al. 1982). However, the energy cost of Na^+/K^+-transport has only been derived for skeletal muscle of sheep and cattle; estimates for the energy cost of Na^+/K^+ transport is lacking for visceral organs such as the liver. In the liver of the rat, Na^+/K^+-transport accounts for 8-31% of the total liver O_2 consumption (Ismail Beigi et al. 1979; Clark et al. 1982; Van Dyke et al. 1983). However, similar estimates have not been derived for species of agricultural importance. Therefore, it was our purpose in this experiment to quantify the energetic cost of ion transport in isolated hepatocytes from growing lambs.

The wide range of values for the energy cost of Na^+/K^+-transport in rat liver (8-31% of the total liver O_2 [illegible] al. [illegible]

Van Dyke et al. 1983), may have arisen through procedural differences resulting in liver and cell preparations of very different viabilities. For this reason, we also studied the effect of viability of the cell preparation on the magnitude of Na^+, K^+-ATPase-dependent respiration or ouabain-sensitive respiration. To our knowledge, there are no estimates of the energy cost of ion transport in the liver of sheep and, similarly, there appear to be no estimates of the effect of viability of the cell preparation studied on the magnitude of ouabain-sensitive hepatocyte respiration.

B. Materials and Methods

Suffolk lambs, 1 wk (5.2 ± 1.2 kg), 3 wk (7.4 ± 1.9 kg) or 8 wk (21.4 ± 3.8 kg) of age, were used in the study. One lamb of each sex was used for each age group. Additionally, hepatocyte preparations of poor viability were derived from two female lambs and one male lamb, 8 wk of age. The lambs were still nursing at this age, although they did have free-choice access to a concentrate creep ration. The lambs 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 (ID 0.86 mm, OD 1.27 mm) was inserted 1 cm into the vein cranial to the ligature. The perfusion procedure outlined by Seglen ([illegible]) was adapted for the lambs. Immediately upon catheterization of the portal vein, the vena cava was severed to facilitate drainage of blood from the liver and a Ca^{2+}-free, gassed (95% O_2/5% CO_2) modified Hank's buffer (Moldeus et al. 1978; pH 7.4 ± 0.1, 0.5 mM EGTA, 26 mM $NaHCO_3$) maintained at 37°C was pumped through the liver at a rate of 100-125 ml/min for 3[illegible] min. During this time, the liver was freed from the body ca[illegible] and was transferred onto surgical gauze stretched across the mouth of a 1000 ml beaker holding 250 ml of gassed (95% O_2/5% CO_2) modified Krebs Henseleit buffer (Dawson et al. 1969; pH 7.4 ± 0.2, 37°C) containing 5 mM Ca^{2+}, 1 mg/ml collagenase IV (Sigma Chemical Co., St. Louis, MO) and 20 mM Hepes. The perfusion intake was switched to the collagenase buffer, which was recirculated through the liver for 15-20 min at a rate of 100-125 ml/min until rupture of the liver outer membrane occurred.

Cells were freed with a stainless steel comb (Seglen, 1976) and collected in gassed (95% O_2/5% CO_2) Krebs Henseleit isolation buffer (Dawson et al. 1969; pH [illegible] ± 0.01) containing 2.9 mM Ca^{2+}, 2% fatty acid poor bovine serum albumin (Sigma Chemical Co.), 20 mM Hepes and 10 mM D-glucose. The isolation procedures were conducted with [illegible] buffers stored on ice. The cell suspension was filtered through nylon mesh (250 µm pore size) and the subsequent filtrate was centrifuged (50 x g, 30 s). The sedimented cells were washed once, resuspended (1 x 10[illegible] cells/ml) in [illegible] Krebs Henseleit [illegible] with continuous gassing (95% O_2/5% CO_2). The total volume yield of cells per liver varied from 200-250 ml at 1 x 10^5 cells/ml.

Cellular damage was assessed by trypan blue uptake into the cell and the leakage of lactate dehydrogenase (EC 1.1.1.27) (LDH) from the cytosol. The percentage of viable cells, as determined by counting the percentage of trypan blue stained cells in an improved Neubauer counting chamber was assessed after storage on ice for 0, 1, 2 and 3 h following isolation. For the determination of LDH leakage, cells (25 mg dry wt) were incubated in 16 ml of gassed (95% O_2/5% CO_2) Krebs-Henseleit isolation buffer stored on ice. Aliquots of 1 ml were removed at 0, 1, 2 and 3 h after isolation. The samples were centrifuged at 50 x g for 1 min and LDH activity in the supernatants was measured by the method of Caud and Wroblewski (1958). Total LDH activity of the cell suspensions was determined in the supernatants of homogenized cell preparations.

In three preparations of poor viability, the same perfusion and isolation procedures as outlined were followed. However, the duration of time between ligation and catheterization of the portal vein was not completed as rapidly as [illegible] resulted in [illegible] viability.

An aliquot of 10 ml (1 x 10[illegible] cells/ml) of isolated hepatocytes was incubated 20 min at 37°C in 90 ml of gassed [illegible] Henseleit isolation buffer [illegible]

0.01) containing 1 mg/ml hyaluronidase III (Sigma Chemical Co.). The purpose was to cleanse the cell surface of adherent mucopolysaccharide (Hayat, 1981). This final cell preparation was centrifuged at 50 x g for 30 s, then the sedimented cells were resuspended in Krebs Henseleit buffer (pH 7.4 ± 0.01) at a concentration of 1 x 10^6 cells/ml. The final cell suspension was continuously gassed (95% O_2/5% CO_2) and stored on ice. Aliquots of this suspension were fixed after 0 and 3 h of storage. Cells were prepared for microscopy by a modification of the technique described by Sanders et al. (1975). The wash and suspension buffer (pH 7.4, 4°C) contained 63.2 mM Na_2HPO_4 [illegible] H_2O and 15.0 mM NaH_2PO_4 H_2O. Isolated cells were washed three times and resuspended at a concentration of 1 x 10 [illegible] cells/ml. A 1 ml aliquot of this suspension was added to 10 ml of 1% glutaraldehyde and fixed for 24 h at 4°C. The cells were washed twice and post fixed in 20 ml of 1% OsO_4 for 2 h at 4°C. Following OsO_4 fixation, the cells were washed, incubated for 10 [illegible] in 1 [illegible] then washed three times and resuspended (1 [illegible] cells/ml, [illegible]). A 50 [illegible] portion of this suspension was applied to a glass coverslip coated with poly-L-lysine hydrobromide [illegible] 70,000-150,000, Sigma Chemical [illegible] St. Louis, MO). The coverslip was mounted in [illegible] retainer ring to allow for convenient handling. The hepatocytes were subsequently dehydrated in a series of graded [illegible] 100% amyl acetate [illegible]

1981). The cells were then critical point dried in CO_2 (Anderson, 1951), coated with gold-palladium and examined under a Cambridge Stereoscan 180 scanning electron miscroscope at a voltage of 20 kv.

O_2 uptake of the hepatocytes was measured polarographically in a Yellow Springs Instrument (YSI) model 53 O_2 electrode assembly. A 100 ul aliquot of the cell suspension (0.8 1.2 mg cell dry wt) was introduced into the electrode chamber containing 4 ml of air saturated Krebs Henseleit isolation buffer (pH 7.40 + 0.01, 37°C) containing 2% fatty acid poor bovine serum albumin, 20 mM Hepes and 10 mM D glucose. Initial O_2 consumption was measured for 15 min, then ouabain was injected into the chamber to give a final concentration of 1 x 10^{-4}M. The O_2 consumption of the ouabain treated cells was measured for a further 20-30 min. The difference between the initial and ouabain insensitive respiration was taken to represent the Na , K ATPase dependent respiration. All respiration rates were expressed on a dry cell weight basis. To measure dry weight the [illegible] on a 1.2 um pore size Millipore [illegible] suspension [illegible]

All data were analyzed by analysis of variance and the [illegible] compared (P<0.05) by either [illegible] multiple [illegible]

C. Results and Discussion

Trypan blue uptake and LDH leakage measurements indicate that the isolated lamb hepatocytes remain viable for up to 3 h of storage on ice (Table IV.1). Change in viability, as measured by trypan blue uptake, did not occur (P>0.05) for cells stored for up to 3 h. These measurements can be compared with values of greater than 95% rep[illegible] rat hepatocytes isolated by similar liver perfusion techniques (Berry and Friend, 1969; Seglen, 1973). The viability measurements of 92.[illegible] [illegible] exceed the mean viabilities previously reported for [illegible] isolated from lambs (8[illegible]%; Clark [illegible] sheep [illegible] and Pogson, 19[illegible]).

The percentage of LDH that leaked from lamb hepatocytes increased from 3.3 to 7.1% [illegible] 3 h [illegible] storage, the percentage of [illegible] from [illegible] cells did not change (P [illegible]) [illegible] there was [illegible] (P<[illegible]) [illegible] at [illegible] In comparison with hep[illegible] f[illegible] [illegible] LDH leakage [illegible] reported [illegible] (5 [illegible] 8.0%; E[illegible] and Pog[illegible] [illegible] Additionally, the LDH leakage [illegible] 17.1) was less than mean [illegible] hepatocytes isolated from [illegible]

...ects of prolonged storage on ice on ... amb hepatocyte viability

		...ation of storage		
				4
...cytes	...	0 ± 1 .4a	... a	.9 5 ±1 4a
...age ...(al)	...	... a b	... ±1 .a b	±1 3b

...tinuously oxygenated hepatocyte preparations were stored on ice for 3 h following ...ion. Percent viability was measured as the percentage of cells that did not uptake trypan ...e leakage of LDH activity into the isolation medium was expressed as a percentage of the ... activity of the cell preparation. Data represent mean ± S.E. for 8 cell preparations.

...s within rows followed by different letters are significantly (P<0.05) different cell damage than trypan blue uptake although the two are considered to [illegible] indicative of only severe plasma membrane damage (Seglen, 1972; B[illegible] et al., [illegible]).

Plates I[illegible] [illegible] and [illegible] b are scanning electron micrographs of lamb hepatocytes [illegible] for [illegible] respectively. The cells appear [illegible] with respect to surface [illegible] [illegible] [illegible] [illegible] [illegible] micrographs [illegible] and [illegible] to electron [illegible] means of evaluating [illegible] presented [illegible] of [illegible]

[illegible]

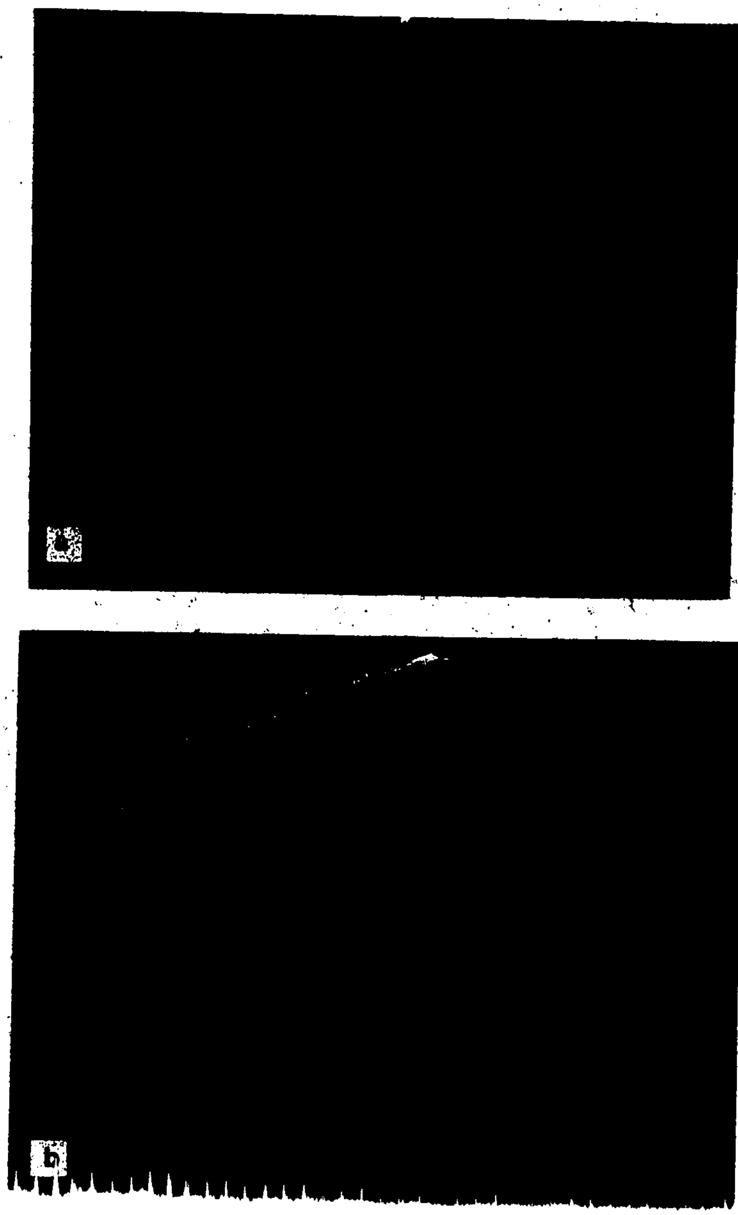


Plate IV.1a,b. Scanning electron micrographs of lamb hepatocytes mounted on polylysine-coated glass coverslips. (a) A lamb hepatocyte fixed immediately after isolation (0 h of storage). The microvilli projections from the cell surface are particularly prominent. Bar= 4 μm. (b). A lamb [illegible] h of storage [illegible] The surface [illegible]

, 2 ˜e effects of amb age on the respiration parameters measured for isolated hepatocytes.*

	...umption ... ..mg dry wt/min)	...nition ...bain	...bain ...ensitive ...spiration ...ol O./mg dry wt/min)	Ouabain ...nsensitive ...espiration nmol O./mg dry wt/min)
	... ±0 23	...2.5	...3 ±0.19	...71 ±0.17...
	... ±0.38	... ±4 5	...7 ±0 33	.58 ±0 28

...en consumption was measured for 100 ul aliquots of the heptocyte preparation suspended in 4 ml of incubation buffer ... ...e). Consumption of the ouabain-treated (1 x 10 M) cells was termed ouabain-insensitive respiration ... epresent means ± ... ...e number of determinations for each age group are in brackets following the age of the ...s

...ns within columns are not significantly P>0.05) different of rats (Webster, 1981) compared to nursing lambs (Degan and Young, 1982) and young sheep (Webster, 1981). The total O_2 consumption rates of the lamb hepatocytes were lower than those previously reported for hepatocytes from lambs of a similar age (8.17-9.10 nmol O_2/mg dry wt/min; Clark et al. 1976), however Clark et al. (1976) used an O_2-saturated buffer for their O_2 uptake measurements. In the present study, an air saturated buffer (37°C, 700 mm Hg, 180 nmol O_2/ml; Umbreit et al. 1964) was used in all O_2 consumption measurements. Higher O_2 utilization rates may be expected from hepatocytes incubated in O_2 saturated buffers, since studies using adult rat hepatocytes have shown higher O_2 uptake rates in O_2 saturated buffers (11.3-11.4 nmol/mg dry wt/min; Ismail-Beigi et al. 1979; Clark et al. 1982) compared to values obtained for rat hepatocytes incubated in air saturated buffers (6 [illegible] nmol O_2/mg dry wt/min; Van [illegible] et al. 1983).

Ouabain sensitive respiration accounted for some 53% of the total O_2 uptake of hepatocytes (Table IV.2 and IV.3). There have not previously been literature reports of the ouabain sensitive respiration of isolated ovine hepatocytes. Estimates of ouabain sensitive respiration range [illegible] of the total respiration of rat liver slices (Ismail-Beigi and Edelman, 197[illegible]; Bernstein et al. 197[illegible]; [illegible] 197[illegible]) and activity of Na⁺,K⁺-ATPase [illegible] of [illegible] O_2 consumption [illegible]

[illegible] 7;

.ie IV 3 The effect of amb hepatocyte viability on respiration parameters

::::ty :::s	:al O_2 .sumption nol O_2/mg dry wt/min)	Inhibition by Ouabain %)	Ouabain -ensitive espiration nmol O_2/mg dry wt/min)	Ouabain -insensitive Respiration (nmol O_2/mg dry wt/min)
? ±2 0(2)	? ±0 60(8)a	5.3 ±5.5a	57 ±0.42a	2.15 ±0.40a
±: 4(2)*	4 ±0 9(12)b**	15 8 ±2.4b	16 ±0 08b	:.48 ±0.18a

-atocyte preparations of different viabilities were isolated from 4 wk old ambs using the same perfusion techniques.
.a represent means ± S.E followed by the number of determinations in brackets

Means within a column followed by different letters are significantly (P<0.01) different

- third preparation was assessed as <50% viable

-e data includes measurements from the three hepatocyte preparations having poor viability

Van Dyke et al. 1983). It has been suggested that procedural or preparation differences may account for some of these differences between estimates of the magnitude of ouabain-sensitive respiration (Folk and Soestoft, 1977; Ismail-Beigi et al. 1979; Clark et al. 1982). However, disparate estimates have still resulted with the use of isolated cells; as noted earlier, from 8-31% of the total respiration of hepatocytes isolated from normal or hyperthyroid rats (Ismail-Beigi et al. 1979; Clark et al. 1982; Van Dyke et al. 1983) was sensitive to ouabain.

Van Dyke et al. (1983) have shown, using both perfused liver and isolated hepatocytes, that Na^+, K^+-ATPase-dependent respiration accounts for approximately one third of the total O_2 consumption. Our results for lambs (Table IV.2 and IV.3) also show that, in viable isolated lamb hepatocytes, the maintenance of Na^+, K^+-ATPase is a major component of cellular energy expenditure. The measurement that 53% of O_2 uptake is to support Na^+, K^+-ATPase activity in hepatocytes isolated from lambs (Table IV.2) is greater than estimates reported for hepatocytes isolated from adult rats (Ismail-Beigi et al. 1979; Van Dyke et al. 1983). These results may reflect a species difference. Gregg and Milligan found the contribution of ouabain-sensitive respiration to total respiration in skeletal muscle to be higher for sheep and cattle (Gregg and Milligan 1982a,b) than for mice (Gregg and Milligan, [illegible] have an influence on ouabain-sensitive respiration because Gregg and Milligan (1982c) reported a 20% greater ouabain-sensitive respiration of sternomandibularis muscle from lambs 2 wk of age, compared to adult control ewes. The literature data available for the contribution of Na^+, K^+-ATPase-dependent respiration to the total O_2 consumption of hepatocytes isolated from rats are entirely for adults (Ismail-Beigi et al. 1979; Clark et al. 1982; Van Dyke et al. 1983); values for young rats would be expected to be greater.

The results presented in Table IV.3 may provide insight into the differences between reports in the literature for the magnitude of ouabain sensitive respiration. The percent viability of hepatocytes isolated from 8 wk-old lambs quite clearly influenced ($P<0.05$) respiration parameters (Table IV.3). The preparations with viability of less than 50% exhibited lower total and ouabain-sensitive respiration. The decrease in ouabain-sensitive respiration entirely accounted for the drop in total respiration (Table IV.3). Clark et al. (1982) concluded that ouabain sensitive respiration accounts for an insignificant proportion (8-15%) of total rat hepatocyte O_2 consumption and heat production. Our results lead to a contrasting conclusion. Aside from differences of species and of physiological development, one would now need to be concerned about the viability of the cell preparation studied. An exact comparison between studies of percent viability cannot be made since Clark et al. (1982) did not [illegible] percentage of LDH leakage was greater (9.5 and 16.7%) than that found for our lamb hepatocytes (Table IV.1).

Ouabain-sensitive respiration has often been equated with Na^+, K^+-ATPase-dependent respiration (Ismail-Beigi et al. 1979; Clark et al. 1982). However, the magnitude of ouabain inhibition of respiration may depend upon the sensitivity of Na^+, K^+-ATPase to ouabain (Schwalb et al. 1982). Since this was not measured, the exact proportion of the total Na^+, K^+-ATPase activity inhibited by ouabain was not known. Therefore, even though the ouabain-sensitive respiration measurements in the present study show that (Na^+ + K^+) transport accounts for approximately 50% of the energy expenditure of lamb hepatocytes, this may be considered a minimal estimate of Na^+, K^+-ATPase-dependent respiration. Furthermore, it is evident that the viability of the cell preparation greatly influences the measurement of the magnitude of ouabain-sensitive respiration, with damaged preparations not being responsive to ouabain.

V. Magnitude of Ouabain-Sensitive Respiration in the Liver of Growing, Lactating and Starved Sheep

A. Introduction

A great deal of research has been directed toward estimating the nutritional efficiency (see Milligan, 1971) of feed conversion to the animal products of meat (Van Es, 1980) or milk (Moe, 1981). The nutritional efficiencies of feed conversion to animal products are usually less than estimates predicted by stoichiometric relationships. For example, the theoretical ME costs for protein synthesis in mammals ranges from 79-88% (Van Es, 1980); however, these estimates are much less than values for nutritional efficiency of 40-60% derived in energy balance or comparative slaughter trials (Kielanowski, 1976; Bullar and Webster, 1976). This disparity between estimations results from an oversimplification of the stoichiometric expression of energetic efficiency. Traditionally energetic efficiency is calculated as the energy content of the synthesized product divided by the energy content of the precursors plus the energy required for synthesis; no account is made for background or maintenance expenditures such as protein turnover and ion transport which are ongoing cellular energy expenditures. These background energy expenditures maintain cellular homoeostasis, yet do not directly yield synthesized products. As stated, stoichiometric calculations of energetic efficiency usually do not account for [illegible] consuming processes such as the turnover of cell constituents which, in the case of whole body protein, may turnover at a rate of 3-8% per d (Waterlow et al. 1978). Degradation of protein to amino acids does not directly yield energy and subsequent resynthesis requires energy. Therefore, the maintenance of cellular homoeostasis includes events which are energetically wasteful in the context of stoichiometrically derived energetic efficiency. Another major maintenance event which utilizes energy, yet may be considered energetically wasteful, is ion transport. This is the cellular energy required to maintain Na^+ and K^+ gradients across the plasma membrane against their concentration gradients (Glynn and Garlish, 1975). The sodium pump (Na^+, K^+ ATPase; EC 3.6.1.3) maintains ionic homoeostasis within cells with the expense of 1 ATP for every 3 Na^+ extruded from the cell and 2 K^+ pumped into the cell (Mandel and Balaban, 1981). This process may account for up to 30-70% of the total energy expenditure of animal tissues, particularly tissues such as liver, gut epithelium, kidney and skeletal muscle ([illegible]erman et al 1979; Balaban et al 1980; Gregg and Milligan, 1982a,b; Van Dyke et al. 1983). Milligan (1971) suggested energy expenditure on ion transport may not be constant in the tissues of animals under different physiological states. The purpose of this experiment, therefore, was to define the energy cost of ion transport in the liver of growing and mature sheep. [illegible] this study was conducted to determine if the maintenance cost of ion transport changes in relation to the physiological demands imposed by lactation or starvation.

B. Experimental

Experiment 1

Animals

Five Suffolk ewes, 2-3 years of age (62.4 ± 1.9 kg), bearing twin lambs, were housed individually with their lambs in claiming pens (3.0 m²) through 8 wk of lactation and for 2 wk following lactation. The ewes were fed twice daily a total of 1.24 ± 0.04 kg/d of both rolled barley (93% dry matter (DM), 11.4% crude protein (CP), 19.0 kJ/g gross energy (GE)) and chopped bromegrass hay (95% DM, 11.1% CP, [illegible] kJ/g gross energy (GE)). This level of feeding was maintained throughout lactation and during the dry period. Water and trace mineralized salt were offered ad libitum.

Milk yield was assessed at 4 wk and 8 wk of lactation using an [illegible] hand-milking procedure. Between 0800 and 0900 h on the day of collection an intramuscular injection of [illegible] 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 [illegible] to prevent suckling [illegible]

[illegible]

Liver biopsy procedure

The liver biopsy method used was similar to that described by Pearson and 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 12 cm ventral to the backbone was clipped and prepared for aseptic insertion of the "Tru Cut" biopsy needle (Travenol Laboratories, St. Louis, MO). The biopsy area was infiltrated with 2% lidocaine, 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 liver puncture was verified by laparotomy in other ewes before the commencement of the experiment. All biopsies were taken between 2-3 h after the morning feeding. The liver biopsies (2-4 mg dry weight) were approximately 5-[illegible] long, [illegible] mm wide and [illegible] than [illegible] mm thick. [illegible] of the liver samples they were washed in [illegible] Krebs-Henseleit buffer (Dawson et al., 196[illegible], pH 7.4 [illegible] 0.01), sliced free hand to less than 0.5 mm [illegible] with a micro[illegible] blade, then incubated in Krebs-Henseleit buffer (Dawson et al., 196[illegible], pH 7.4 + [illegible] 37 C) containing 2[illegible] [illegible] pooled [illegible] in [illegible] M [illegible] before being transfe[illegible] to the oxyge[illegible] chamber.

Hepatocyte isolation and viability

Hepatocytes were isolated from one of the twin lambs from each ewe at 4 wk of age (11.[illegible] ± 1.[illegible] kg) [illegible] from the remaining lambs at 5 wk of age (20.[illegible] ± 1.5 kg). They were removed from their dams immediately before surgery and were therefore not fasted at the time of liver perfusion. The surgical and liver perfusion technique [illegible] to the [illegible] hepatocytes was ide[illegible] Chapter IV.

Hepatocytes were [illegible] non pregnant, dry ewes [illegible] which [illegible] chopped [illegible] liver was [illegible] using the method [illegible] excision [illegible] perfused [illegible] major vessel [illegible] rpm, [illegible]

Neubauer counting chamber and expressed as a percentage of the total cell number.

O_2 consumption and ouabain sensitive and insensitive respiration measurements

O_2 uptakes of the liver tissues and hepatocytes were measured polarographically in a Yellow Springs Instrument (YSI) model 53 O_2 electrode ass[illegible] 10 min of preincubation in air saturated Krebs-Henseleit buffer (pH 7.40 ± 0.01, [illegible]) [illegible] aliquot of the hepatocyte cell suspension was introduced into the electrode chamber [illegible] sat[illegible] (pH 7.[illegible] [illegible] [illegible] 15 min [illegible] to [illegible]. The [illegible] cons[illegible] treated [illegible] measured for [illegible] The di[illegible] initial [illegible] [illegible] [illegible] calculated [illegible] respiration over [illegible] [illegible] responding [illegible] [illegible] concentrations of 1 x 10^{-4}M ouabain.

Measurements of ouabain-sensitive $^{86}Rb^{+}$ uptake

The rates of $^{86}Rb^{+}$ uptake in hepatocytes from an 8 wk old lamb were measured in 1.9 ml of a gassed (95% O_2/5% CO_2) Krebs-Henseleit incubation buffer (pH 7.40 ± 0.01, 37°C) containing 2% fatty acid poor bovine serum albumin, 20 mM Hepes, 10 mM D-glucose, 2.5 μCi/ml $^{86}Rb^{+}$ (New England Nuclear) and 0.1 mM RbCl. Aliquots of 100 μl of the hepatocyte preparation were added to incubation buffer containing [illegible] M ouabain. Ouabain sensitive $^{86}Rb^{+}$ uptake was measured in duplicate samples treated with [illegible] M ouabain [illegible] 10 min in shaking water bath [illegible] Total [illegible] uptake was determined [illegible] the same [illegible] untreated hepatocytes. The difference [illegible] total [illegible] uptake [illegible] uptake of the [illegible] hepatocytes [illegible]

[illegible]

[illegible] ouabai[illegible] course of [illegible] filtration of the cells onto polycarbonate filters (8 μm pore size, Nucleopore, Minneapolis, MI). The cells and filters were washed three times with ice cold phosphate-buffered saline (pH 7.40 ± 0.01, 10 mM NaH_2PO_4 H_2O, 0.85% NaCl, 0.1 mM RbCl), transferred to 15 ml plastic scintillation vials and digested in 1 ml of Protosol (New England Nuclear) at 55°C for 20 min. Glacial acetic acid (50 μl) was added to decolour the samples then 10 ml of Unisolve I scintillation fluid (Terochem Laboratories Ltd., Edmonton, ALB) was added to the vials. The samples were immediately counted on a Nuclear Chicago Mark I scintillation counter using balance point counting with a 20:1 dynamic range window

Measurements of ^3H ouabain binding

Total ouabain binding to hepatocytes isolated from two 8 wk old lambs was determined in 1.9 ml of gassed (95% O_2/5% CO_2) Krebs-Henseleit incubation buffer (pH 7.40 ± 0.01, 37°C) containing 2% bovine serum albumin, 20 mM Hepes, 10 mM D glucose and 0.5 $\mu Ci/ml$ ^3H-ouabain (1[illegible] Ci/mmol, New England Nuclear). Aliquots of 100 μl of the cell suspension were added to buffers containing 10[illegible] – 10[illegible] M ouabain and were incubated for 30 min in a shaking water bath. The cells were filtered onto polycarbonate filters (Nucleopore) and washed three times with ice cold phosphate-buffered saline (pH 7.40 [illegible] mM NaH_2PO_4 H_2O, 0.85% NaCl). The filters and cells were transferred to 15 ml plastic scintillation vials, 1 ml of Protosol (New England Nuclear) was added and the cells and filters were digested for 20 min at 55°C. Glacial acetic acid (50 μl) was added followed by the addition of 10 ml of Unisolve I scintillation fluid (Terochem Laboratories Ltd.). The β emissions from the samples were then counted in a ^{3}H-window of a Searle Analytic Mark III scintillation counter using an external standard pulse height to determine counting efficiency.

The difference between total ^{3}H-ouabain binding at 30 min and non-specific ^{3}H-ouabain binding at 10^{-3} M-ouabain was taken to represent specific ouabain-binding (Akera and Cheng, 1977). Specific ^{3}H-ouabain binding was then used to determine the number of ^{3}H-ouabain binding sites using Scatchard analysis (Scatchard, 1949).

Experiment 2

Animals

Four Suffolk wethers all weighing 50 kg and 1 y of age were fed 1.0 kg/d of chopped bromegrass hay (95% dry matter, 14% crude protein, 16.6 kJ GE/g) in two equal allotments twice (0800 and 1600) daily to achieve maintenance. Another group of four 1 y old Suffolk wethers (49 ± 1 kg) were starved for 5 d. Both groups of animals had free access to trace-mineralized salt and water.

Whole animal O_2 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 O_2 consumption were made with the fed animals. The sheep were subjected to surgery on the morning of the 6th day of starvation or 3 h after the morning feeding.

Hepatocyte isolation and measurements

The caudate lobe perfusion procedures described for adult ewes in Experiment 1 were used for hepatocyte isolati[illegible]n consumption, ouabain-sensitive respiration (1×10^{-4} M-ouabian) and ouabain-insensitive respiration rates were measured for hepatocytes isolated from the fed and starved sheep as described in Experiment 1. Similarly, all ouabain-sensitive $^{86}Rb^+$ uptake and ouabain binding measurements were made for hepatocytes, isolated from two fed and starved sheep, as outlined in Experiment 1.

Analysis of results

Respiration rates, $^{86}Rb^+$ uptakes and ^3H-ouabain binding were expressed on a dry weight basis; to measure dry weight, hepatocytes, which had been filtered onto 1.2 μm pore size millipore filters, and liver biopsies were dried at 90°C for 12 h. All data were analyzed 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 and Torrie, 1960).

C. Results

Experiment 1

Ouabain inhibition of O_2 and $^{86}Rb^+$ uptake

The response curves for ouabain-inhibition of O_2 and $^{86}Rb^+$ uptake by lamb hepatocytes are shown in Fig. V.1. The sigmoidal nature of ouabain inhibition of both O_2 and $^{86}Rb^+$ uptake was similar. The lowest concentration of ouabain yielding maximum inhibition of O_2 consumption was 10^{-4} M while the lowest concentration of ouabain which permitted maximum inhibition of $^{86}Rb^+$ uptake by lamb hepatocytes was 10^{-5} M.

Ouabain inhibition of $^{86}Rb^+$ uptake in hepatocytes was immediate (Fig. V.2). Within 1 min of exposure to 10^{-4} M-ouabain, 48.2 ± 4.6% of the $^{86}Rb^+$ uptake by hepatocytes had been inhibited. At 5 min of exposure to ouabain, the $^{86}Rb^+$ uptake of lamb hepatocytes had been inhibited by 68.1 ± 0.9%. This measurement was not different ($P>0.05$) from the maximum inhibition of 80.5 ± 6.9% attained at 30 min. Peak inhibition of $^{86}Rb^+$ uptake by hepatocytes was maintained from 5 to 60 min of incubation (Fig. V.2).

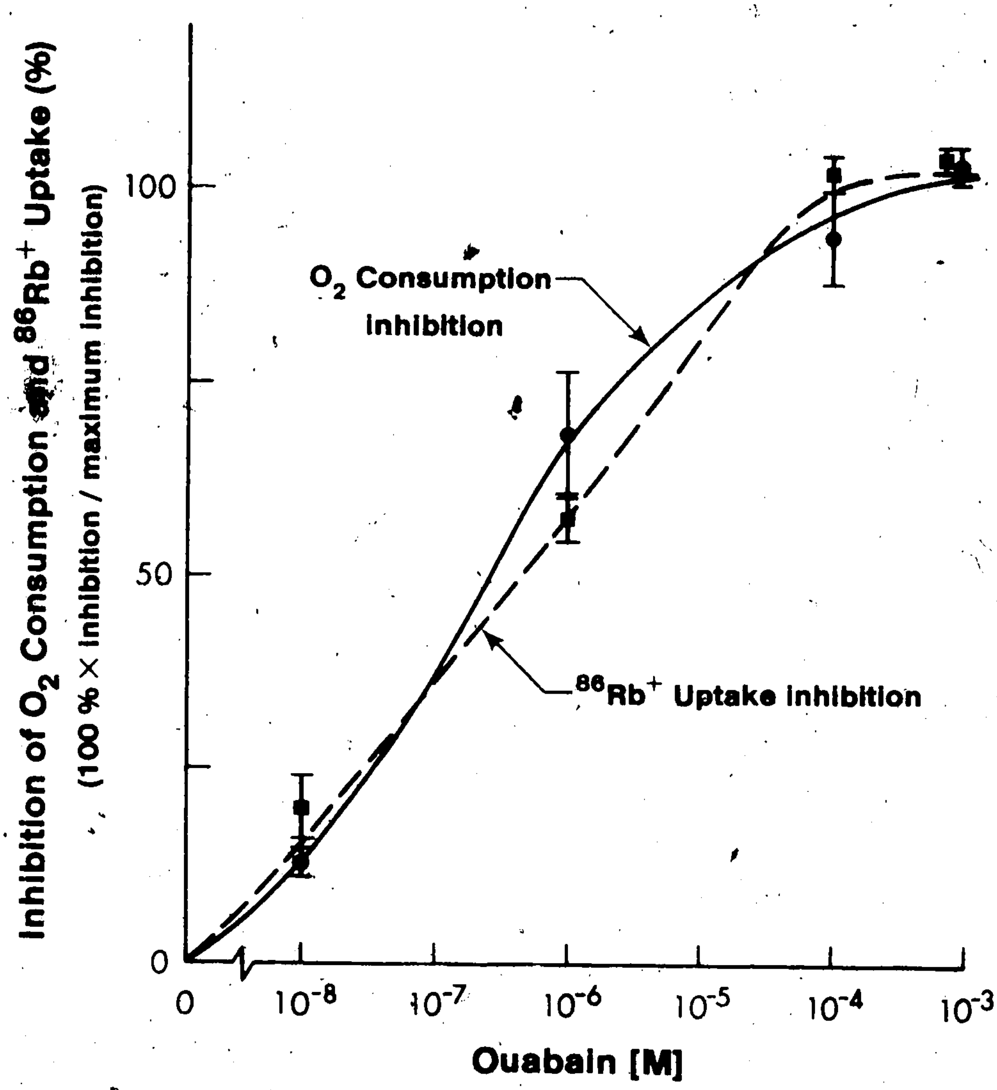


Figure V.1.
Inhibition of lamb hepatocyte O_2 consumption and $^{86}Rb^+$ uptake by ouabain. Inhibitions are expressed as a percentage of maximum inhibition. Values are means ± S.E.

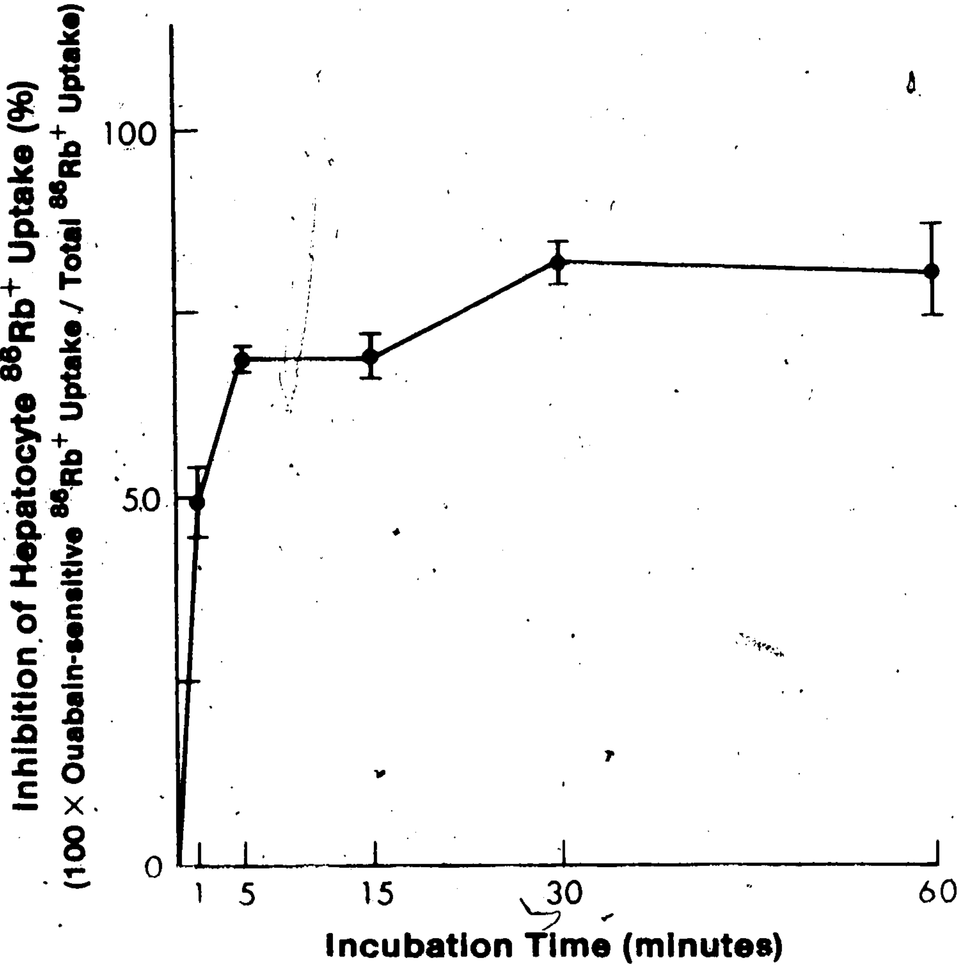


Figure V.2.
Time course of inhibition of lamb hepatocyte $^{86}Rb^{+}$ uptake by ouabain (1×10^{-4} M). Values are means ± S.E.

Ouabain binding

Total ouabain binding to lamb hepatocytes in increasing concentrations of ouabain is shown in Fig. V.3. The level of total ouabain binding to hepatocytes increased linearly on a log-log plot with increased ouabain concentration. Scatchard analysis of specific ^3H-ouabain binding data resulted in an estimation of 1.97 ± 0.77 pmoles/mg cell dry wt of ^3H-ouabain binding sites to lamb hepatocytes.

O_2 uptake and ouabain-sensitive respiration

The results of the effect of lactation on respiration parameters and milk production are shown in Table V.1. Total milk production dropped ($P<0.05$) by 42% from week 4 to week 8 of lactation. The accompanying respiration responses measured in the liver paralleled the decrease in the animals' metabolic demand as exemplified by milk production. Total O_2 consumption rates of the liver biopsies were slightly lower ($P>0.05$) in non-lactating compared to lactating ewes (Table V.1). During peak lactation, the ouabain-sensitive respiration of the liver biopsies accounted for 45% of the total tissue O_2 consumption. This measurement was 1.24-1.37 times those made during late lactation and during the dry period. The magnitude of ouabain-sensitive respiration in the liver of ewes at peak lactation (1.48 ± 0.15 nmol O_2/mg/min) was also 29-43% higher ($P<0.10$) than similar measurements made

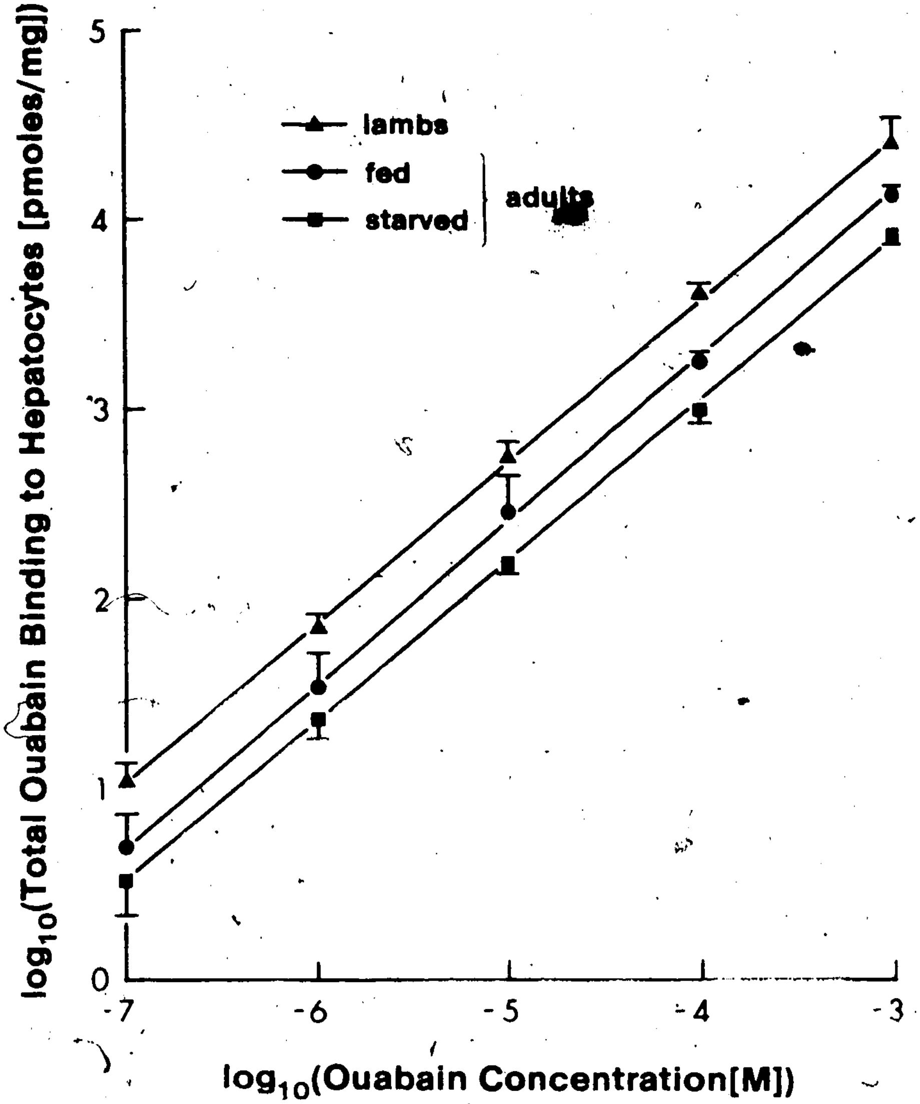


Figure V.3.
Log of total ouabain binding to hepatocytes isolated from 8 wk old lambs and adult sheep fed to maintenance or starved 5 days plotted against the log of the ouabain concentration. The incubation period was 30 min. Values are means ± S.E. The equations for the lines are:

$\log_{10}Y = 0.839 \log_{10}X + 6.924$ r=0.997 (▲ - lambs)
$\log_{10}Y = 0.857 \log_{10}X + 6.699$ r=0.989 (● - fed adult sheep)
$\log_{10}Y = 0.845 \log_{10}X + 6.440$ r=0.994 (■ - starved adult sheep)

Y = total ouabain binding to hepatocytes (pmoles/mg)
X = ouabain concentration in the medium [M]

Table V.1. Milk production of lactating ewes and O_2 consumption parameters from liver biopsies of lactating and non-lactating ewes.*

Physiological state (n)	Milk production (g/d)		Total O_2 consumption (nmol O_2/mg/min)		Percent inhibition of O_2 consumption by ouabain		Ouabain-sensitive respiration (nmol O_2/mg/min)**		Ouabain-insensitive respiration (nmol O_2/mg/min)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
lactating										
4 wk (10)	2252	252*a*	3.36	0.31*a*	45.1	3.8*a*	1.48	0.15*a*	1.88	0.25*a*
8 wk (9)	1305	198*b*	3.16	0.27*a*	32.9	4.2*b*	1.04	0.12*b*	2.12	0.26*a*
non-lactating (9)			3.14	0.43*a*	36.5	3.0*b*	1.15	0.20*b*	1.99	0.28*a*

* All respiration results are expressed on a mg dry weight basis.

** Means within this column followed by different letters are significantly ($P<0.10$) different.

a,b Means within columns followed by different letters are significantly ($P<0.05$) different.

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 preparations from the mature sheep were 11 percentage units lower ($P<0.05$) than measurements of 90-93% determined for lamb hepatocytes; however, the magnitude of this difference was much less than the differences of 40-70% determined for respiration parameters between age groups.

The physiological effects of animal age on total hepatocyte O_2 consumption and ouabain-sensitive respiration are shown in Table V.2. All respiration measurements for hepatocytes isolated from lambs aged 4 and 8 wk were similar ($P>0.05$). Total hepatocyte O_2 consumption of the mature sheep was also much less ($P<0.05$) than that found for lambs (Table V.2). The O_2 consumption rates of hepatocytes from mature sheep were 39-46% lower ($P<0.05$) than values of 4.86-5.62 nmol O_2/mg/min observed for lambs (Table V.2). Additionally, the magnitude of ouabain-sensitive respiration of hepatocytes isolated from mature sheep was 67-69% lower ($P<0.05$) than similar measurements made for lamb hepatocytes. The only respiration parameter of the sheep hepatocytes that did not differ ($P>0.05$) with respect to the animals' age was

Table V.2. Growth rates of lambs and O_2 consumption parameters of hepatocytes isolated from mature sheep and infant lambs.*

Age (n)	Average daily gain (g/d)		Hepatocyte viability (%)		Total O_2 consumption (nmol O_2/mg/min)		Percentage inhibition of O_2 consumption by ouabain		Quabain -sensitive respiration (nmol O_2/mg/min)		Ouabain -insensitive respiration (nmol O_2/mg/min)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
4 wk(5)	285	40*a*	93	2*a*	5.62	0.28*a*	47.8	[illegible].8*a*	2.67	0.21*a*	2.95	0.31*a*
8 wk(4)	230	41*a*	90	2*a*	4.86	0.22*a*	51.0	3.0*a*	2.50	0.19*a*	2.36	0.17*a*
3 y(2)			80	1*b*	3.00	0.10*b*	27.9	2.6*b*	0.82	0.07*b*	2.18	0.14*a*

* All respiration results are expressed on a mg dry weight basis.

a,b Means within columns followed by different letters are significantly ($P<0.05$) different.

ouabain-insensitive respiration (Table V.2). 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.

Experiment 2

$^{86}Rb^+$ uptake measurements

The dose-response curves of ouabain-inhibition of $^{86}Rb^+$ uptake of hepatocytes from fed and starved sheep are shown in Fig. V.4 The sigmoidal pattern of ouabain-inhibition of hepatocyte uptake of $^{86}Rb^+$ was similar for both fed and starved sheep and identical to the response curve observed for lambs. The lowest ouabain concentrations, causing maximum inhibition of $^{86}Rb^+$ uptake, were 10^{-4} M and 10^{-5} M for hepatocytes of fed and starved sheep, respectively. The time course of ouabain-inhibition of hepatocyte-$^{86}Rb^+$ uptake, for fed and starved sheep, is shown in Fig. V.5 The magnitude of ouabain-sensitive $^{86}Rb^+$ 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 $^{86}Rb^+$ uptake by hepatocytes from fed sheep was three to nine times greater ($P<0.05$) than by cells from starved sheep (Fig. V.5). As was found with lamb hepatocytes, maximum percentages of

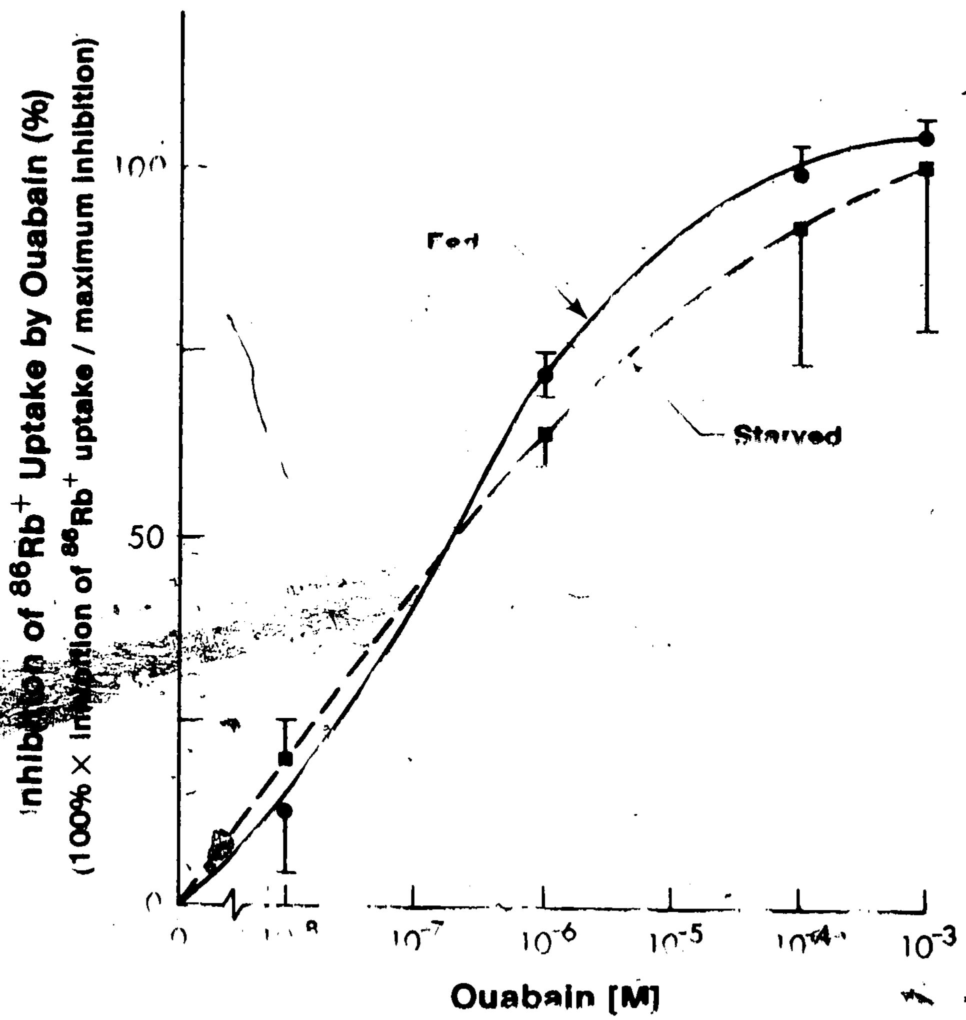
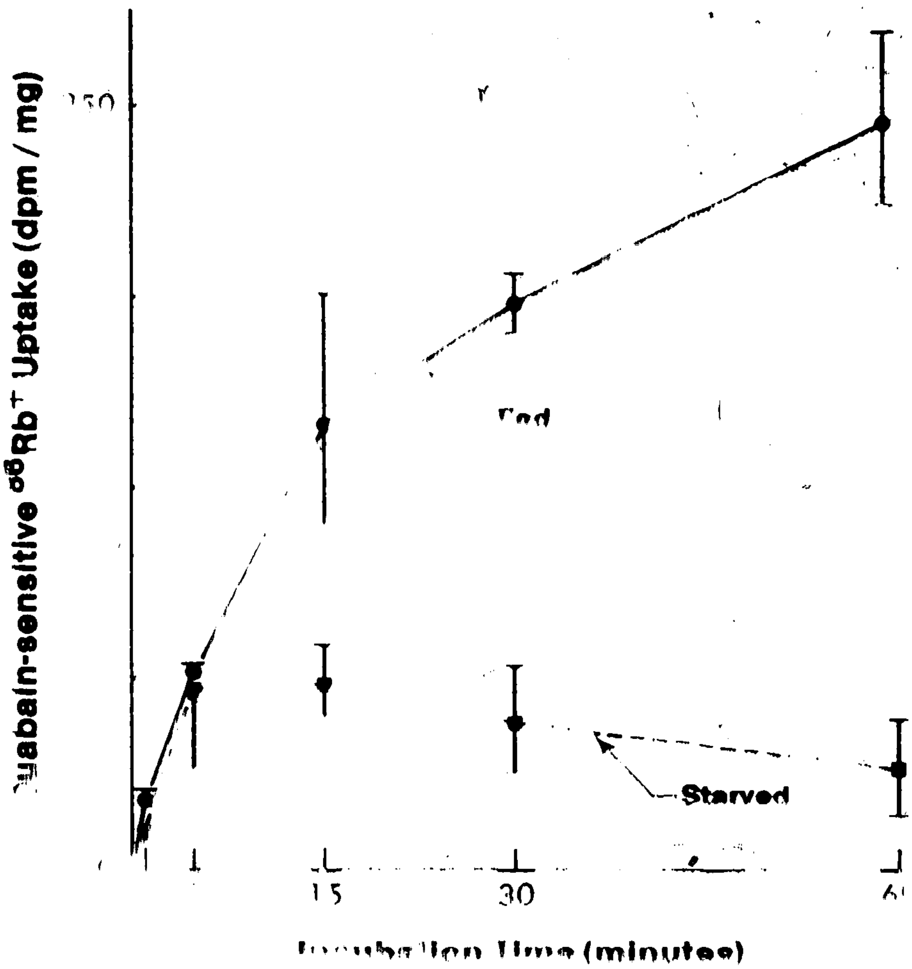


Figure V.4.
Inhibition of hepatocyte $^{86}Rb^+$ uptake by ouabain. The response curves for fed and starved sheep. Values are means ± S.E.



ab... sensitive ... uptake of hepatocytes ouabain inhibition of hepatocyte-$^{86}Rb^+$ uptakes were attained within 5 min of exposure to 10^{-4} M-ouabain.

Ouabain binding

Total ouabain binding to hepatocytes, isolated from fed and starved sheep, is shown in Fig. V.3. In both fed and starved sheep, total ouabain binding to hepatocytes increased ($P<0.01$) with increasing concentration of ouabain. Total ouabain binding to hepatocytes was greater ($P<0.0$[illegible]) for lambs than adult sheep and greater ($P<0.07$) for fed adult sheep than for starved adult sheep (Fig. V.3). The maximum number ouabain binding sites of hepatocytes from fed and starved sheep, as determined by Scatchard analysis were 0.654 ± 0.191 pmoles/mg cell dry wt and 0.193 ± 0.135 pmoles/mg cell dry wt. These values were not significantly ($P>0.05$) different from each other [illegible] from the values obtained for lamb hepa[illegible]

Whole animal and hepatocyte respiration

Whole animal O_2 consumption rates and hepatocyte respiration rates are [illegible] in Table V.3. [illegible] O_2 consumption rates were lower [illegible] ($P<0.05$) for starved sheep compared to the fed sheep. This [illegible] also [illegible] for total hepatocyte O_2 consumption [illegible] hepatocyte preparations from starved sheep had [illegible]%

[illegible]

..e / 3. Whole animal O_2 consumption and hepatocyte O_2 consumption parameters of adult fed and starved sheep.*

..ological ..te	Whole animal consumption (..l O_2/kg/h)		..epatocyte total O_2 consumption (nmol O_2/mg/min)		Percentage inhibition of O_2 consumption by ouabain		Ouabain -sensitive respiration (nmol O_2/mg/min)		Ouabain -insensitive respiration (nmol O_2/mg/min)		Hepatocyte viability (%)	
	..an	..E	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
.. (4)	..3	..3	..02	0.18*a*	41.1	4.1*a*	..27	0.15*a*	1.75	0.10*a*	90.5	2.3*a*
..rved	..5	..)	..13	0.44*a*	17.8	7.1*b*	0.48	0.20*b*	1.65	0.26*a*	97.8	2.3*a*

..respiration results are expressed on a mg dry weight basis.

..eans within a column followed by different letters are significantly ($P<0.05$) different.

(90.5 and 97.8%) were not different ($P>0.05$) from one another (Table V.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 62 % lower ($P<0.05$) than for hepatocytes from fed sheep; the drop in ouabain-sensitive respiration accounted for 89% of the observed decrease in total hepatocyte O_2 consumption due to starvation. The ouabain-insensitive component of hepatocyte O_2 consumption did not differ ($P>0.05$) between preparations from fed and starved sheep (Table V.3).

D. Discussion

Ouabain-sensitive respiration rates reported in this study provide only an estimation of Na^+, K^+-ATPase-dependent respiration, since the exact proportion of total Na^+, K^+ ATPase activity inhibited by ouabain was not measured. Even considering this constraint, ouabain-sensitive respiration of both lamb and adult sheep hepatocytes and liver biopsies of mature sheep accounted for a major proportion of total cell and tissue O_2 consumption. During peak lactation, ouabain sensitive respiration of liver biopsies [illegible] was accounted for 45% of the total tissue O_2 consumption. This proportion corresponded with the highest [illegible] in the liver of lactating ewes. It has been suggested that elevated Na^+, K^+-ATPase activity may occur in the liver to support the 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. Our results suggest that the maintenance energy costs required to support Na^+/K^+-transport in the liver increases during peak lactation. Stimulation of Na^+, K^+-ATPase by thyroid hormones may provide an explanation for elevated ouabain-sensitive respiration rates determined for the livers of lactating ewes. Elevated plasma concentrations of T_3 are found in lactating cows (Bines and Hart, 1978; Trenkle, 1978) and T_3 is reported to increase the activity and number of Na^+, K^+-ATPase units in mammalian liver (Ismail-Beigi, 1970; 1971; Lin and Akera, 1978). Additionally, higher ouabain-sensitive respiration has been found in the skeletal muscle of lactating sheep compared to values obtained from non-lactating sheep (Gregg and Milligan, 1982c). The results of this study and that by Gregg and Milligan (1982c) suggest that more maintenance energy may be necessary to support Na^+, K^+-ATPase activity in liver and skeletal muscle during peak lactation as compared to the non-lactating state.

As stated, the respiration parameters for lamb hepatocytes were similar in both 4 and 8 wk old animals. Total O_2 consumption rates of the lamb hepatocytes were lower than those previously reported for hepatocytes from lambs of a similar age (Clark et al. 1976); however, these workers used an O_2-saturated buffer for their O_2 uptake measurements. In our study, an air-saturated buffer was used in all O_2 consumption measurements. Studies using adult rat hepatocytes have shown higher O_2 uptake rates in O_2-saturated buffers (11.3-11.4 nmol O_2/mg dry wt/min; Ismail-Beigi et al. 1979; Clark et al. 1982) compared to values obtained for rat hepatocytes incubated in air-saturated buffers (6.62-9.48 nmol O_2/mg dry wt/min; Van Dyke et al. 1983). Furthermore, the O_2 consumption rates determined for lamb hepatocytes were within the range of O_2 uptake rates determined for lamb liver in situ (4.74-10.68 nmol O_2/mg dry wt/min; Edelstone and Holzman, 1981).

Ouabain-sensitive respiration of lamb hepatocytes accounted for approximately 50% of the total hepatocyte O_2 consumption. This parameter was twice as large as that determined for hepatocytes isolated from mature sheep. Similarly, total hepatocyte O_2 consumption was 62-87% greater for growing lambs compared to mature sheep. The greater ouabain-sensitive respiration of lamb hepatocytes accounted for 71-90% of the greater hepatocyte O_2 consumption observed between growing lambs and mature sheep. We have found greater total ouabain binding to lamb hepatocytes compared to those found for hepatocytes isolated from mature wethers, although the activity of individual Na^+, K^+-ATPase units tended to be lower for lamb hepatocytes compared to those isolated from mature sheep (1.26 vs 1.94

nmoles O_2/pmole ouabain binding site/min). Other work has also shown the number of Na^+, K^+-ATPase units are greater in the liver of younger animals compared to those found in older animals (Lin et al. 1979a,b). These results suggest that higher maintenance energy expenditures are necessary to support Na^+, K^+-ATPase activity in hepatocytes isolated from growing lambs compared to mature sheep. This finding is also consistent with the observation that mature animals have a lower overall maintenance energy expenditure than young growing animals (Moe, 1981; Webster, 1981) especially considering that the liver contributes up to 10-15% of the total heat production of animals (Lautt, 1976; 1977; Edelstone and Holzman, 1981).

It is also apparent from this 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 ion transport than those from starved sheep. Background or maintenance energy expenditure on hepatic ion transport, therefore is likely not constant in animals at different feeding levels.

The change in the magnitude of ouabain-sensitive respiration of hepatocytes due to starvation may reflect alterations in either the number or activity of Na^+, K^+-ATPase units in the cells. Total ouabain-binding to hepatocytes and the number of ^3H-ouabain binding sites of hepatocytes tended to be higher for fed sheep compared to starved sheep. Ouabain-sensitive $^{86}Rb^+$ uptake, after 15 min of incubation, by hepatocytes from fed sheep was also considerably higher than those values obtained from starved sheep. It appears that fed sheep have greater ouabain-sensitive $^{86}Rb^+$ uptake and respiration in hepatocytes than starved sheep in response to an increase in the number of enzyme units per cell, although the activity of individual Na^+, K^+-ATPase units tended to be lower for hepatocytes isolated from fed sheep compared to starved sheep (1.94 vs 2.49 nmoles O_2/pmole ouabain binding site/min).

Ouabain-insensitive respiration of hepatocytes represents a measure of all cellular energy expenditures other than the energy costs of ion transport; therefore, it includes the energy cost of cellular syntheses. In this study, the animals' lactational state, age and feeding level had no significant effect on the magnitude of ouabain-insensitive respiration of isolated hepatocytes and liver biopsies. This would suggest that the energy cost to support the synthesis of rapidly turning over cell constituents in the liver remains unchanged during these physiological states.

The results of this study show that ouabain-sensitive respiration accounts for approximately 50% of total in vitro liver O_2 consumption of rapidly growing lambs and lactating ewes. More energy is expended in support of ion transport in young growing animals and animals in peak lactation than in mature animals held at maintenance intakes. Furthermore, the maintenance energy expenditure associated with ion transport in hepatocytes markedly changes with the feeding level of sheep. The drop due to starvation in total hepatocyte O_2 consumption was nearly entirely accounted for by a drop in ouabain-sensitive respiration. Lastly, the energy costs associated with the maintenance of other cellular processes did not significantly change in hepatocytes isolated from growing and mature sheep or fed and starved sheep.

VI. General Summary and Conclusions

This study showed that Na^+/K^+-transport accounts for a major component of duodenal mucosa energy expenditure. In sheep, maintenance of Na^+/K^+-transport costs between 28.6 - 61.3% of total duodenal mucosa energy expenditure. These values are approximately 35% higher than similar estimates reported for support of Na^+, K^+-ATPase in small intestinal mucosa of rats (Liber[illegible] et al. 1979). The lower ouabain inhibition of rat mucosal [illegible], K^+-ATPase may represent a species difference in ouabain susceptibility since previous work indicates that higher concentrations of ouabain are required to reach maximal inhibition of O_2 consumption in rats compared to dogs, sheep or cattle (Tobin and Brody, 1972).

In sheep fed 7.6 MJ digestible energy per day, Na^+, K^+-ATPase-dependent respiration accounted for 50% of the total O_2 consumption of duodenal mucosa. This result was highly repeatable in the same animals, as evidenced by similar measurements obtained 3 months later. This estimate of Na^+, K^+ ATPase-dependent respiration was also duplicated in mucosal biopsies incubated in Na^+ free media. The agreement between estimates of Na^+, K^+-ATPase dependent respiration derived from use of ouabain as a specific inhibitor of Na^+, K^+-ATPase or with the use of Na^+ free media suggested that these estimates were valid measures of Na^+, K^+ ATPase respiration and did not arise from altered intracellular [illegible] of Na^+

The magnitude of Na^+, K^+-ATPase-dependent respiration of duodenal mucosa was influenced by the animals' feed intake. Na^+, K^+-ATPase-dependent respiration of duodenal mucosa increased by 37% when sheep were fed higher digestible energy intakes and decreased by 45% in sheep starved for 48 h. Lower energy expenditure on ion transport during intervals of feed deprivation may provide a mechanism to conserve energy.

The magnitude of energy required to support ion transport in duodenal mucosa of cows also changes during lactation. Na^+, K^+-ATPase-dependent respiration of duodenal mucosa accounted for 55% of total mucosal O_2 consumption of cows at peak lactation. In mid-lactation and during the non-lactating period, the proportion of O_2 uptake inhibited by ouabain declined to 34 - 35%. Elevated ouabain-sensitive respiration may be representative of an increase in synthesis of Na^+, K^+-ATPase during peak lactation in response to increased thyroid activity in lactating cows.

This study showed that ouabain-sensitive respiration accounted for 15.8 - 55.3% of lamb hepatocyte O_2 consumption. In lamb hepatocyte preparations with viabilities greater than 90%, ouabain-sensitive respiration accounted for 52.4 - 55.3% of total cell respiration. Lamb hepatocyte preparations with viabilities less than 50% exhibited lower total and ouabain-sensitive respiration. The decrease in ouabain-sensitive respiration in these preparations accounted entirely for the drop in total hepatocyte respiration. It was evident that the viability of the cell preparation greatly influenced the measurement of ouabain-sensitive respiration, with damaged preparations not being reponsive to ouabain.

The magnitude of ouabain-sensitive respiration of hepatocytes and liver biopsies was also dependent upon the animals' physiological state. Ouabain-sensitive respiration of liver biopsies from ewes in peak lactation accounted for 45% of the total O_2 consumption of the biopsies. This proportion was 1.24-1.37 times higher than similar measurements made during late lactation and during the non-lactating period. Total O_2 consumption and ouabain-insensitive respiration of the liver biopsies from ewes were unaffected by the lactational status of the ewes.

The age of the animal influenced the cellular energy expenditure on ion transport. Total hepatocyte O_2 consumption rates of mature sheep were 30 40% lower than values observed for 4 and 8 wk old lambs. The magnitude of ouabain sensitive respiration of hepatocytes isolated from mature sheep was 67 69% lower than similar measurements for lamb hepatocytes. Ouabain sensitive respiration accounted for 48 51% of total O_2 consumption of hepatocytes isolated from infant lambs but only accounted for 28% of the total O_2 uptake of hepatocytes isolated from mature sheep. The decrease in the [illegible] ATPase activity in hepatocytes of mature sheep [illegible] explained by [illegible] ouabain [illegible] enzyme [illegible] cell.

As found with duodenal mucosa, the animals' feed intake also influenced the activity of hepatic Na^+, K^+-ATPase. Ouabain-sensitive $^{86}Rb^+$ uptake of hepatocytes from starved sheep was 56 - 78% lower than measurements obtained from hepatocytes of fed sheep. Similarly, the magnitude of ouabain-sensitive respiration of hepatocytes from starved sheep was 62% lower than those values found for hepatocytes of fed sheep. The drop in ouabain-sensitive respiration accounted for 89% of the observed decrease in total hepatocyte O_2 consumption due to starvation. The ouabain-insensitive component of hepatocyte O_2 uptake did not differ between hepatocytes isolated from fed and starved sheep. The decrease in Na^+, K^+-ATPase activity in hepatocytes isolated from starved sheep was partially explained by a decrease in the number of total ouabain binding sites per cell.

Support of Na^+, K^+ transport in duodenal mucosa and hepatocytes of sheep and cattle accounts for a major but variable proportion (15.8 - 61.3%) of total cellular energy expenditure. The magnitude of cellular energy expended on this metabolic event appears dependent upon the feed intake [illegible] of the animal.

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VIII. Appendix 1: Development of a Technique for Gastrointestinal Endoscopy of Domestic Ruminants.[1]

A. Introduction

The first clinically useful fibre-optic endoscope was constructed by Curtiss et al. (1957) and used by Hirschowitz et al. (1958) at the University of Michigan Hospital. However, common clinical use of endoscopy was not realized until 1968 (Salmon, 1974). Endoscopic assessment of gastrointestinal tract disorders and abberations in humans is a routine procedure (Belber, 1971; Salmon et al. 1971; Cotton et al. 1972; Blumgart and Salmon, 1973), but has not been applied extensively to the study of domestic animals. Ehrlein (1979) made reference to endoscopic observations in a report emphasizing a radiological study of digesta flow through the forestomachs of goats however photographic accounts of these observations were not presented. Similarly, there appear to be no published reports of intestinal endoscopy with domestic ruminants. This investigation was undertaken to develop a technique of gastrointestinal endoscopy in sheep and cattle and to explore the normal physiological events associated with digestion in the ruminant.

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[1]A slightly modified version of this chapter has been published. McBride, B.W., Berzins, R., Milligan, L.P. and Turner, B.V. (1983). Can. J. Anim. Sci. 63, 349-354.

B. Materials and Methods

Cannulae and Animal Preparation

To allow the placement of endoscopes to specific areas of the gastrointestinal tract, cannulae were inserted in the descending duodenum of sheep and the rumen and proximal duodenum of a steer. The mature Holstein steer and a yearling wether were fitted with two duodenal cannulae spaced approximatelly 20 cm (wether) or 30 cm (steer) apart in the descending duodenum to permit examination of the luminal side of the cannula and surrounding mucosa. A 10-cm rumen cannula (Bar Diamond Inc., Parma, ID) and 'T'-type cannulae (ID 25 mm) made of silicone rubber (W.C. Ellis, Texas A&M University, College Station, TX) were used for the steer. The internal diameters of these cannulae allow entry and passage of the endoscope into the gastrointestinal tract of cattle. For sheep it was necessary to construct a cannula specifically for endoscopy because the available 'T'-type cannula with adequate internal diameter was too large to be inserted into the duodenum. The design incorporates a minimal luminal flange to reduce irritation of the intestinal mucosa and a peritoneal ring to secure the cannula to the body wall. The cannula was turned in a lathe from a Delrin nylon rod to the specifications shown in Plate VIII.1. The barrel was threaded to allow a screw fit with the threaded nylon nut, inset in a dome-shaped plastisol (F.H. and Sons Manufacturing Ltd., Concord, Ontario) ring.

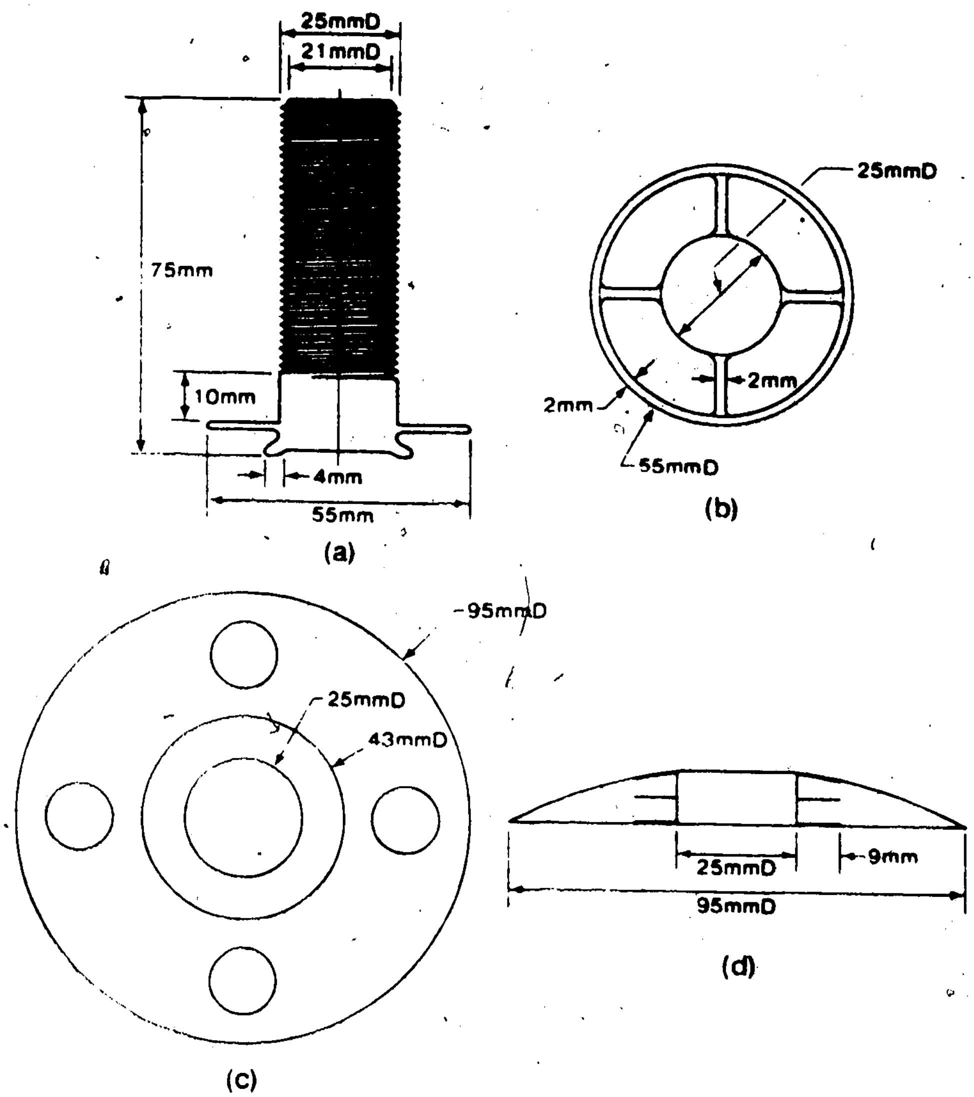


Plate VIII.1. Delrin nylon intestinal cannula and associated components: (a) side view of intestinal cannula showing the threaded barrel (b) cannula top view showing peritoneal ring (c) top view of plastisol ring with embedded nylon nut (d) side view of plastisol ring.

Surgical Procedures

During surgery on eight sheep, general anesthesia was induced and maintained with halothane gas. The abdomen was opened with a lateral incision distal to the ribs and duodenum was located. The beginning of the descending duodenum was opened and the cannula was secured in position with a purse-string suture. The barrel of the cannula was exteriorized through a stab wound between the 12th and 13th ribs. The dome-shaped cap was threaded flush with the body wall and the barrel of the cannula was cut to a length even with the dome-shaped cap. The outer ring of the cannula held it firmly in place and also prevented the animal from lying directly on an exposed cannula barrel. The lumen of the cannula was plugged with a rubber septum.

Endoscopy Equipment and Procedure

Two endoscopes were utilized in this study. An ACMI colonoscope (OD 15.8 mm) model 9000-F (American Cytoscope Makers, Inc., Stamford, CT) was used with the sheep and steer whereas, an Olympus colonoscope (OD 18 mm) model TCF Type 2L (Olympus Corporation of America, New Hyde Park, N.Y.) was used exclusively with the steer. An Olympus CLK-3 cold light source, having a colour temperature of 3200°K, was used for all photography. Air feeding and distal lens washing were achieved through the porting system of the endoscopes using either an attached air pump and a syringe or the air pumping system (2,000 cc/min at 0.18 kg/cm²) of the light source.

The endoscopy procedure was performed while the animals were standing, conscious and in a fed state. A mature Holstein steer (528 kg) was fed 6-kg of long alfalfa hay twice (0800 and 1600h) daily. The 8 yearling wethers, averaging 44±2 kg, were fed a maintenance (947±29 g/d) diet of alfalfa pellets in two equal allotments (0800 and 1600 h). Water and salt were offered ad-libitum. During intestinal endoscopy prior evacuation of the intestine was not required. The endoscope was inserted through the intestinal cannula and was visually guided along the intestine.

Immediately before rumen endoscopy the reticulo-rumen was partially emptied of both solid and fluid digesta. The endoscope was hand guided to the desired location of the reticulo-rumen through the rumen cannula. To photograph under fluid in the rumen, Tygon tubing was stretched over the distal end of the endoscope to produce a protruding sleeve which extended 2 cm beyond the endoscope. The end of the extended sleeve was pressed against the rumen wall while air was pumped through the endoscope to create an air pocket in the sleeve. This procedure allowed viewing of the internal rumen surface with limited interference by digesta

Endoscopic Photography

Once the endoscope was positioned, a 35 mm camera was attached to the proximal head of the endoscope with an eyepiece adapter. A Pentax, Spotmatic II, 35 mm camera fitted with a 100 mm bellows lens (Takumar f:4.0) set at infinity was used exclusively with the ACMI colonoscope. With the Olympus colonoscope, the camera back of an OM-2N camera, fitted with an Olympus 1-9 focusing screen, was placed directly onto the proximal head of the colonoscope. Photography was accomplished with the endoscope proximal lens set at a fixed focus. Each system required a specific endoscope to camera adapter (ACMI Lirrmann Adapter and Olympus SM-2S Adapter).

An f stop of 4.0 was selected for all photography with the Pentax camera. Shutter speeds were either 1/30 or 1/60 sec. The films used were either Kodak (ASA 400) Ektachrome slide film or Kodak (ASA 160) tungsten balanced slide film.

Shutter speeds of 1/15, 1/30 and 1/60 sec were employed with the Olympus system. Kodak (ASA 160) tungsten balanced slide film was used exclusively with this system.

All slides were processed using the Kodak E6 process. Prints were generated from slides using the Ciba [illegible] process. This system proved to be less expensive and provided superior print reproduction [illegible] generating [illegible]

Results and Discussion

Various features of the gastrointestinal tract of sheep and cattle were photographed using endoscopy. The photograph [illegible] shows the proximal duodenum of a Holstein

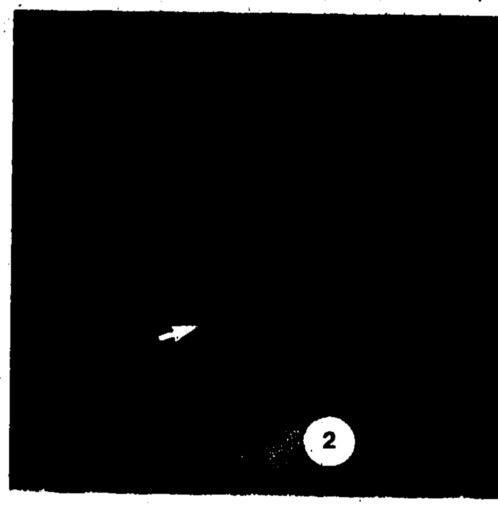
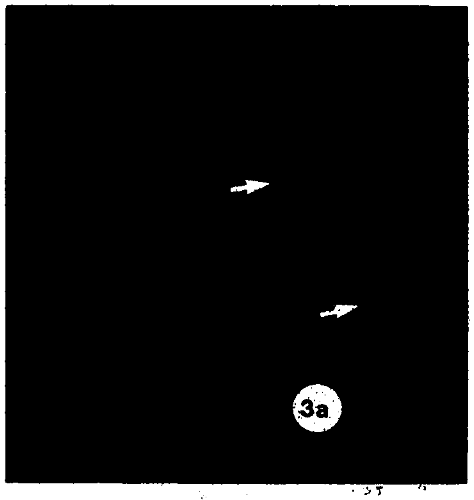


Plate VIII.2. The proximal duodenum of a steer. Kerckring folds in the duodenal wall are evident at the arrow. The photograph was taken while viewing distally down the duodenum.

steer. The Kerckring folds of the duodenal wall were especially evident and were used as an identifying feature of the proximal small intestine during endoscopy in the present study (Beck et al. 1975) The silicone rubber intestinal cannula appeared as a green object in the lumen of the steer (Plate VIII.3a). No observable anatomical lesions were evident in the intestinal epithelium [illegible] areas adjacent to the cannula at 4 weeks after surgery. Furthermore we saw no indications that inhibition of digesta flow was caused by the cannula. Similar observations [illegible] made in sheep using the nylon cannula design (Plate VIII.3b). Wenham (1970) has shown by radiological techniques that [illegible] cannulae provide less inhibition to digesta flow than [illegible] cannula designs but does indicate that [illegible] cannulae with long (35 [illegible]) rigid gutter shaped flanges inhibit complete closure of the intestinal lumen upon contraction. The reduction of the luminal portion of the nylon cannula to a small flange [illegible] and use of a flexible silicone rubber [illegible] cannula appears to have eliminated the [illegible] [illegible]



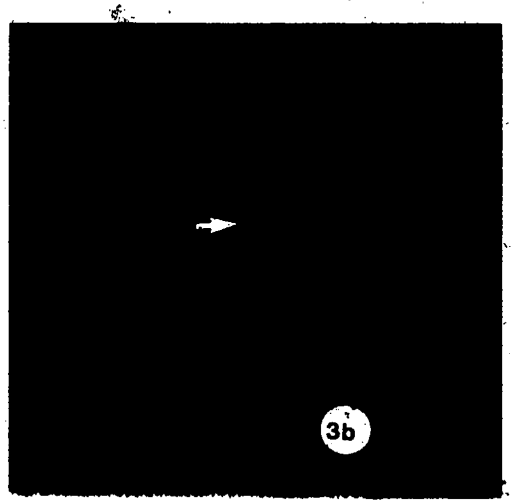


Plate VIII.3a,b. Intestinal cannulae photographed in situ. a) The silicone rubber 'T'-cannula positioned in the proximal duodenum of a steer. The lower arrow points to the luminal portion of the 'T' cannula. The intestine has contracted over the cannula and reduced the diameter of lumen as seen at the upper arrow. b) The Delrin nylon intestinal cannula situated in the descending duodenum of a sheep. The arrow points to the flange of the [illegible]
[illegible] the duodenum

suction biopsy tube (Brandborg et al. 1959) was passed parallel to the endoscope through the intestinal cannula into the descending duodenum of a sheep. The biopsy tube was advanced in front of the endoscope to a specific identifiable location. The intestinal epithelium was drawn into the sampling capsule (Plate VIII.4) and the tissue was excised. Repeated sampling of the same area of the duodenum was possible thus yielding good replication. A similar biopsy method has been reported for humans (Rubin and Dobbins, 1965; Trier, 1971; Rubin et al. 1970).

The endoscope was directed through rumen contents to view the reticulo-omasal orifice (Plate VIII.5a) and omasum (Plate VIII.5b) in a Hostein steer. The endoscope was under digesta when these areas were photographed. In Plate VIII.5a, fluid can be seen entering the open orifice and the unguliform or claw like papillae at the entrance to the orifice are readily apparent. Plate VIII.5b shows the edge of two omasal leaves covered with numerous small horny papillae and a fragmented plant stem lodged between the omasal leaves.

The present work indicates that gastrointestinal endoscopy can be applied to domestic ruminants in a conscious and, more importantly, fed state. Endoscopes designed for use in humans are not long enough to pass from the mouth to intestine of cattle and, while the animal is standing, it would be exceedingly difficult to precisely direct [illegible] lly inserted endoscope in the [illegible]

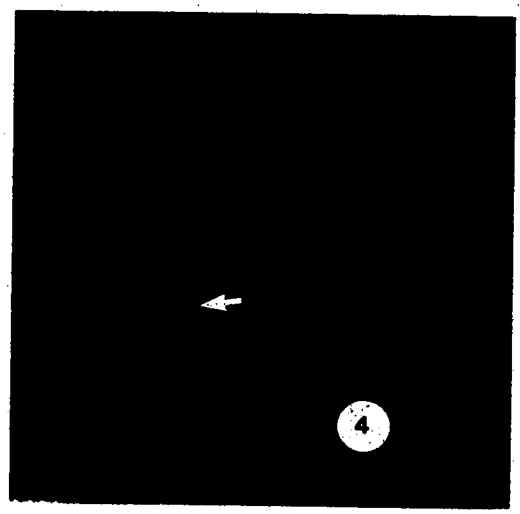


Plate VIII.4. A site specific intestinal epithelium biopsy technique shown in the duodenum of a sheep. The arrow indicates the intestinal wall being drawn into the sampling capsule.

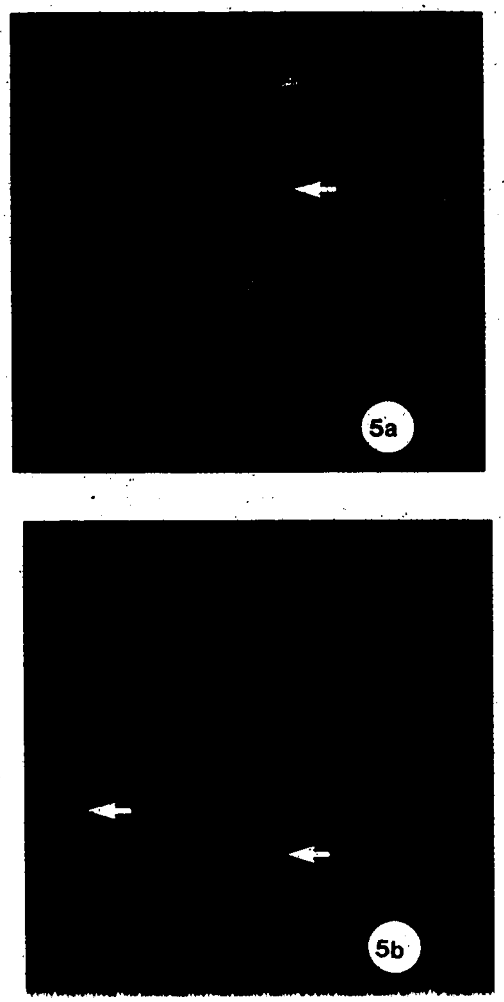


Plate VIII.5a,b. The reticulo-omasal orifice and omasum of a Holstein steer. a) The reticulo-omasal orifice in an open position. The arrow points to an unguliform papilla positioned anterior to the orifice. b) An endoscopic photograph taken of the interior of the omasum. The arrow in the center of the photograph points to a plant stem embedded between two omasal leaves. Several horny papillae are indicated at the arrow to the left of the photograph.

the rumen. As a result, direct access to the gastrointestinal tract was accomplished by means of rumen and duodenal cannulae.

There is now very great interest in the mechanisms and kinetics of passage of the particulate components of digesta through the forestomach and the intestinal tract of ruminants. Researchers are exploring approaches involving use of tracers (Ellis et al. 1979), particle sieving (Poppi et al. 1980) and description of digesta physical properties (Evans et al. 1973; Welch, 1982) as rather indirect means of improving understanding of movement of digesta through the forestomach. Direct endoscopic observation of movements of digesta and physical operation of digestive structures in fed animals may, in itself, result in a more complete understanding of the mechanisms involved in passage of digesta, and will certainly give valuable guidance as to what other measurements and approaches would be most informative. Knowledge of the control of particle movement from the rumen is also important in understanding what limits food intake.

IX. Appendix 2: Endoscopic Observations of Particle Movement into the Reticulo-Omasal Orifice of Cattle.[1]

A. Introduction

A variety of factors influence the voluntary intake of ruminants including reticulo-rumen fill or distension (Campling and Balch, 1961; Welch, 1967 and Grovum, 1979) and rate of passage of digesta through the gastrointestinal tract (Bines and Davey 1970). The exit point of the reticulo-rumen, the reticulo-omasal orifice, potentially serves as a control site in regulation of particle movement from the rumen.

However, little definitive information on the functional behavior of this anatomical location is available. Therefore, a fibre-optic endoscopic technique was designed to allow visual observation of the reticulo-omasal orifice in conscious fed cattle.

B. Materials and Methods

A mature Holstein steer (528 kg) was fitted with ruminal and duodenal cannulae (Bar Diamond Inc., Parma, ID) 6 months prior to endoscopy. Rumen cannulation was performed as described by Dougherty (1981). Long lucerne hay was offered in 6-kg portions twice (0800 and 1600h) daily for 14 d before endoscopy. Water and salt were offered ad-libitum.

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[1] A slightly modified version of this chapter has been published. McBride, B.W., Milligan, L.P. and Turner, B.V. (1983). J. agric. Sci., Camb. 101, 749-750.

The endoscopy procedure was conducted while the animal was standing, conscious and in a fed state. Immediately prior to endoscopy, coarse (hay mat) and fluid digesta (approximately 75 % of the digesta volume) were removed from the dorsal and ventral rumen using a beaker. This permitted hand guiding the endoscope through the rumen cannula to a position anterior to the reticul-omasal orifice. During endoscopy, the reticulum and ventral rumen were under fluid digesta. To photograph under fluid, Tygon tubing was stretched over the distal end of the endoscope to produce a protruding sleeve. This procedure allowed viewing of the reticulo-omasal orifice with limited interference by digesta.

An eyepiece adapter (Olympus SM-2S Adapter) was fitted to the proximal head of an Olympus colonoscope (Model TCF-2L, Olympus Corporation of America, New Hyde Park, NY) and a 35 mm camera (Olympus OM-2N with 1-9 focusing screen) was attached. Photographs were taken at a shutter speed of 1/30 or 1/60 of a second. The film used was Kodak (ASA 160) tungsten balanced slide film. Prints were generated from slides using the Cibachrome P30 process. An Olympus CLK-3 cold light source, having a colour temperature of 3200 °K, provided the illumination for projection through the endoscope. Opening and closing of the reticulo-omasal orifice were photographed using this procedure.

C. Results and Discussion

Plate IX.1 shows gradual opening of the reticulo-omasal orifice. Unguliform or claw-like horny papillae of this anatomical region of the reticulo-rumen are quite distinct (Plates IX.1b,c). An omasal leaf is prominent in Plate IX.1c when the orifice is fully open. In Plates IX.1a-c, the area photographed is aproximately 9 cm^2 when fully open (Plate IX.1c), the orifice was ellipsoid and approximately 45 mm long and 10 mm in width. These are much greater dimensions than the 3-4 mm that is rarely exceeded by particles that reach the lower digestive tract (Van Soest, 1966; Smith et al. 1967; Poppi et al. 1980).

Balch, Kelly and Heim (1951) showed, using fed cattle, that the reticulo-omasal orifice closed during the first phase of reticular contraction and never fully dilated until the reticulum relaxed following the second phase of contraction. Observations of the reticulo-omasal orifice during the contraction sequence of the reticulo-rumen confirmed this result.

The orifice was seen to close in conjunction with the contraction of the reticulum and closure of the reticular groove. The margins of the orifice were observed to roll inwards and fold together upon closing. The orifice opened only while the reticulum was relaxed following its biphasic contraction, the reticulo-ruminal fold was in an upright position and the reticular groove was stretched open. During opening, the margins of the orifice roll outwards to expose

Plate IX.1a,b,c. (a) The reticulo-omasal orifice of a Holstein steer in a closed position. The arrow indicates the location of the closed orifice. (b) The reticulo-omasal orifice folding open, exposing the unguliform papillae. The arrow points to an unguliform papilla. (c) The reticulo-omasal orifice fully open exposing the edge of an omasal leaf. The arrow points to the omasal leaf.

the interior of the omasum. The opening and closing of the orifice was completely unlike the action of the iris diaphragm of a camera.

The results attained in the present study indicate that the technique of fibre-optic endoscopy can be applied to fed, conscious cattle to observe physiological events associated with digestion.

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