反芻動物の消化管におけるモノカルボン酸輸送体1(MCT1)に関する研究

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Monocarboxylate transporter 1 (MCT1) in the gastrointestinal tract of ruminants

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1. Introduction

In ruminants, short-chain fatty acids (SCFA; acetate, propionate and butyrate) are the end products of the anaerobic microbial fermentation of carbohydrates in the gastrointestinal tract. They constitute the major source of energy; providing up to 80% of the maintenance energy requirements in ruminants[1]. Propionate is also the major substrate of hepatic gluconeogenesis in ruminants. In addition to the energetic or nutritional contributions of SCFA to the body, the SCFA help in regulation of endocrine and exocrine secretion of pancreas. Moreover, SCFA production and absorption have a significant effect on epithelial cell growth, blood flow, and the normal secretory and absorptive functions of gastrointestinal tract[1]. Prior to use by the animal, these microbial products must be transferred and effectively absorbed across the gastrointestinal epithelium.

To date, carrier-mediated transport of SCFA in the ruminant gastrointestinal tract has not been conclusively described; and despite the significance of SCFA in maintaining the ruminant physiology, the cellular mechanism(s) for their absorption from the gastrointestinal epithelia are still incompletely known. SCFA are weak monocarboxylic acids with a pKa around 4.8, thus at the physiological pH most of SCFA are present as anions[2]. Therefore, carrier-mediated transport across the epithelial cell membranes seems to be required.

The transport of short chain monocarboxylates across the plasma membrane in most cells is largely dependent on a family of specific MCTs (monocarboxylate transporters; also termed the SLC16 gene family). The MCTs family includes up to fourteen members, each having different tissue distribution and only the first four (MCT1-MCT4) have been demonstrated experimentally to catalyze the proton-linked transport of metabolically important monocarboxylates such as lactate, pyruvate and ketone bodies[3,4]. The differences in the properties of MCT isoforms are related to the metabolic requirements of the tissue in which they are expressed[5]. Amongst the MCT isoforms, MCT1 is expressed most widely and has the broadest substrate specificity; furthermore it is likely to play a central role in metabolism and metabolic communications in many tissues[6].

Although studies on MCT1 carried out have focused exclusively on the human and monogastric animals[3], we have recently conducted, for the first time, a series of studies concerning MCT1 in the gastrointestinal tract of ruminants[7, 8, 9, 10, 11].

In the present work we illustrate the possible involvement of MCT1 in the SCFA transport across the ruminant gastrointestinal tract. To achieve this goal, our efforts were focused to fulfill the following three main aspects: 1. Studying the expression, distribution and the precise cellular localization of MCT1 along the entire length of the gastrointestinal tract in various ruminants including bovine, ovine, and

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caprine using reverse transcriptase polymerase chain reaction (RT-PCR), Western blotting and immunohistochemistry; 2. Studying the co-localization of MCT1 and its chaperone ancillary protein, CD147, using double immunofluorescence confocal laser-scanning microscopy; 3. Exploring the physiological role of MCT1 in the ruminant gastrointestinal tract using both *in vivo* and *in vitro* functional studies on the rumen and cecum epithelium.

2. Expression, Distribution, and Cellular Localization of Monocarboxylate Transporter 1 (MCT1) in the Gastrointestinal Tract of Ruminants

We studied the expression and regional distribution of MCT1, using different molecular biology techniques, on both mRNA and protein levels. Also, we delineated the precise cellular localization and levels of MCT1 protein along the gastrointestinal tract of various ruminant species, including bovine, ovine, and caprine[7,8,9,10,11].

Reverse transcription-polymerase chain reaction (RT-PCR) was carried out as previously described[10,11] to elucidate the expression of MCT1 on the mRNA level. A single cDNA fragment of the expected size was obtained by RT-PCR amplification of total RNA from the different regions of cattle, sheep and goat gastrointestinal tracts (Fig. 1). The product was absent in the negative control. GAPDH was also monitored by RT-PCR as an indicator for the quality of the extracted RNA as well as the cDNA integrity of the various tissues examined. Product specificity was assessed by sequencing the amplified MCT1 cDNA fragments. The nucleotide and amino acid sequences of the PCR products of MCT1 have been deposited to the GenBank under the accession numbers: **AB250265** and **AB231662** for bovine and caprine gastrointestinal tracts, respectively. The homology searches of the identified PCR fragment against the previously published nucleotide sequences of MCT1, demonstrated ~ 85-99% identities with the equivalent regions of different animal species including bovine, ovine, equine, human, Chinese hamster, and rat

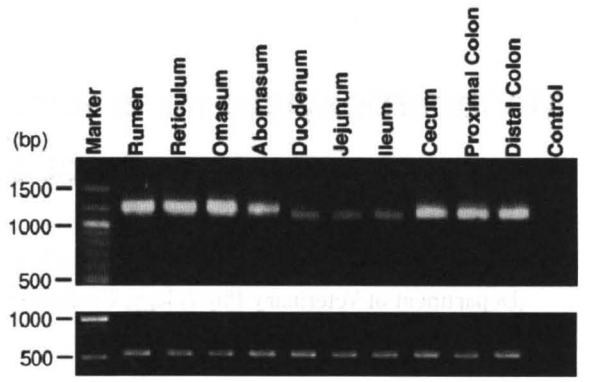

Figure 1. Representative RT-PCR analysis of MCT1 in ruminant gastrointestinal tract.
PCR was performed using MCT1-specific primers and templates consisting of total RNA from the indicated tissues of cattle gastrointestinal tract. The product was separated on 1% agarose gel. RT-PCR yielded an amplified band of the expected molecular size of 1200 bp. No band was observed in the negative control study. GAPDH (~500 bp) was used as an internal standard on all tissue (bottom gels).

MCT1.

Western blot analysis was conducted to investigate the distribution pattern as well as expression level of MCT1 protein along the length of gastrointestinal tract of different ruminant animals[9,10,11]. The anti-MCT1 antibody (chicken anti-rat MCT1; AB1286; Chemicon International Inc., Temecula, CA, USA) recognized a protein migrating with an apparent molecular mass of 43–45 kDa in immunoblots of plasma membrane proteins prepared from the various regions of gastrointestinal tract of bovine, ovine, and caprine (Fig. 2A). Specificity of the immunoreaction was confirmed by preabsorption of the antibody with its corresponding antigen, which resulted in complete blocking of the antigen-antibody reaction.

The relative abundance of MCT1 protein bands, as measured by the densitometric analysis, in the entire regions of the ruminant gastrointestinal tract are shown in Fig. 2B. The MCT1 protein level was found most abundantly in the forestomach, at intermediate levels in large intestine and abomasum, and very low levels in the small intestine. Within stomach, MCT1 protein levels were significantly higher

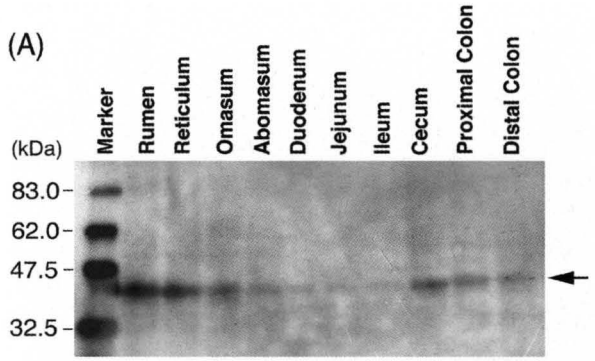

Figure 2A. Western analysis of MCT1 protein content in membrane preparations from different parts of ruminant gastrointestinal tract (represented by caprine).
MCT1 antibody (at a dilution of 1: 1000) recognized a band of 45 kDa. Twenty five micrograms membrane proteins collected from the examined tissues were electrophoresed on 10% SDS-polyacrylamide gel and probed with the MCT1-antibody. Bands were detected using the Enhanced Chemiluminescense System (ECL; Amersham International, UK). [J. Physiol. (London.), 2006, 576: 635-647]

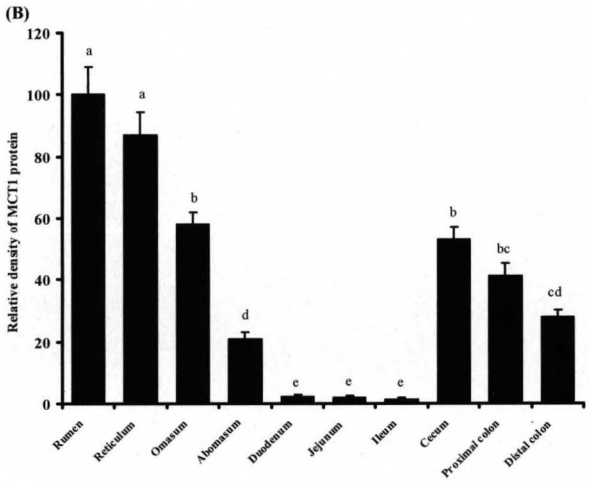

Figure 2B. Representative quantitative densitometric analyses of Western blot for MCT1 protein abundance in all regions of ruminant gastrointestinal tract.
Values are expressed as means ± SE of five different blots. Values with unlike letters are significantly different. [J. Physiol. (London.), 2006, 576: 635-647]

($P < 0.01$) in the rumen and reticulum in comparison with omasum and abomasum, however the relative expression of MCT1 level was much higher in the omasum compared with the abomasum. In the large intestine, MCT1 protein levels were highest in the cecum followed by proximal colon and then distal colon. On the other hand, the MCT1 levels among the duodenum, jejunum, and ileum were not significantly different ($P > 0.05$).

The precise cellular localization of MCT1 protein along the length of ruminant gastrointestinal tract was further studied by immunohistochemical analysis as well as by the immunofluorescence confocal laser-scanning microscopy[9, 10, 11]. Within the ruminant gastrointestinal epithelium, using MCT1 antibody, the immunopositive staining for MCT1 was observed in the stratified squamous epithelial cells of the forestomach compartments as well as in the epithelial cells lining the abomasum and all parts of large intestine, while we could detect weak immunoreactive staining for MCT1 in various regions of the small intestine.

Amongst the stratified squamous epithelial cells of the forestomach (rumen, reticulum, omasum), MCT1 density was predominately expressed in the cell boundaries of stratum basale and stratum spinosum (Fig. 3B-E). In the abomasum of the various ruminants studied, however, MCT1 was localized at the basolateral membranes of the surface epithelial cells (Fig. 3F). Among the ruminant large intestine, MCT1 immunoreactivity was mostly confined to the basolateral membranes of the surface epithelium lining cecum and proximal and distal colon; and the intensity was decreased toward the base of the crypt (Fig. 4A-C). On the other hand, faint immunoreactive staining has been detected for MCT1 in the ruminant small intestine. Negative control preparations exhibited no staining for the MCT1 (Fig. 3A and 4D).

3. Co-localization of Monocarboxylate Transporter 1 (MCT1) with CD147 in the Ruminant Gastrointestinal Tract

The study was also undertaken to examine the co-localization of MCT1 with its chaperone, CD147 in the ruminant gastrointestinal tract using double immunofluorescence confocal laser-

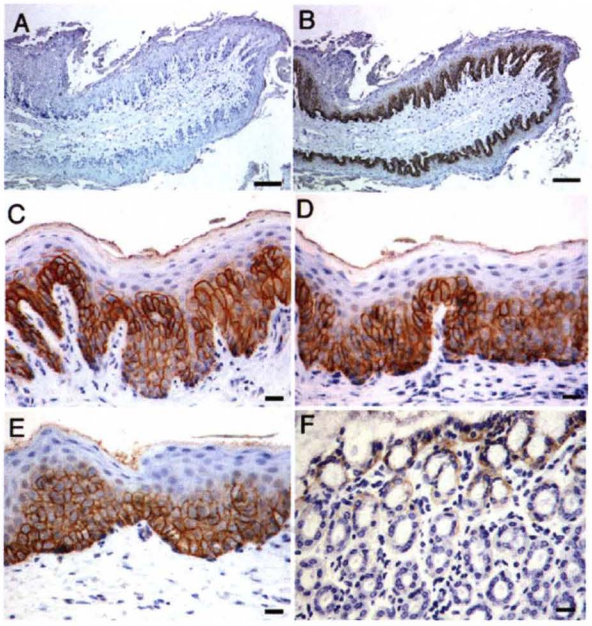

Figure 3. Immunohistochemical images of MCT1 in ruminant stomach (represented by sheep). Sections from sheep rumen (B, C), reticulum (D), omasum (E) and abomasum (F) were fixed, processed, and stained with MCT1 antibody. MCT1-positive cells are labeled in brown. Negative control; section from sheep rumen (A) incubated with MCT1 antibody preabsorbed with its peptide antigen, showing no immunospecific staining for MCT1. Scale bar is 50 mm for images A and B; and 30 mm for all other images. [Vet. J., 2006, 171: 462-467]

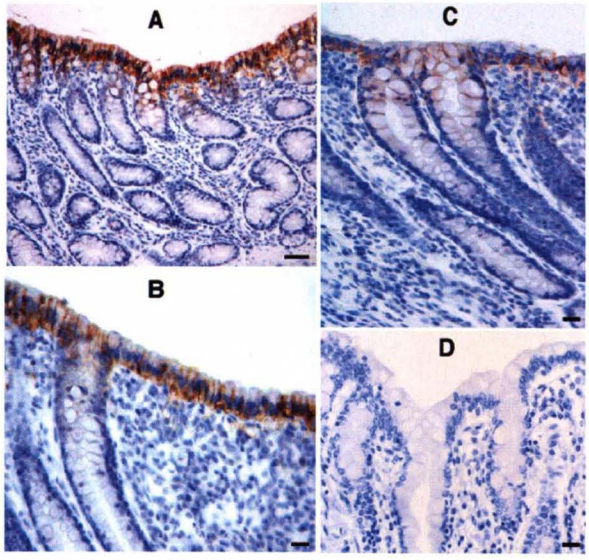

Figure 4. Cellular localization of MCT1 in the large intestine of ruminants (represented by sheep). Sections of cecum (A), proximal colon (B), and distal colon (C) were reacted with the MCT1 antibody. MCT1-positive cells are labeled in brown. Note the prominent membrane staining of MCT1 protein in the basolateral membrane of the surface epithelia of large intestine. (D): Section of cecal epithelia incubated with MCT1 antibody preabsorbed with its peptide antigen, as a negative control, showing no immunospecific staining for MCT1. Immunohistochemistry was performed using the Vectastain Elite ABC kit. Scale bar is 50 mm for image A; and 30 mm for all other images. [Vet. J., 2006, 171: 462-467]

scanning microscopy[10]. Our results showed that the MCT1 chaperon, CD147, was co-localized with MCT1 in the cells of stratum spinosum and stratum basale of forestomach (Fig. 5), as well as in the basolateral membranes of the epithelial cells lining the abomasum and large intestine (Fig. 6). The co-localization of MCT1 with CD147 substantiates the fact that CD147 is required for the proper localization of MCT1 in the ruminant gastrointestinal epithelial cells.

4. Functional Role of Monocarboxylate Transporter 1 (MCT1) in Ruminant Gastrointestinal Tract

In this study we carried out two different experiments to explore the role of MCT1 in the SCFA transport across the ruminant gastrointestinal tract epithelium. The first experiment was conducted using *in vivo* and *in vitro* functional studies for MCT1 transport across the caprine rumen[10], while the second experiment was performed using *in vitro* transport studies across the bovine cecum epithelium[11]. *In vivo* and *in vitro* functional studies, under the influence of the MCT1 inhibitors, *p*-chloromercuribenzoate (pCMB) and *p*-chloromercuribenzoic acid (pCMBA), respectively demonstrated significant inhibitory effect on acetate and propionate transport across the caprine rumen (Fig. 7, 8). The *in vitro* studies on the isolated mucosal sheets of cattle cecum via Ussing chamber technique verified that the transport of acetate across the cecal epithelium was significantly reduced by serosal application of pCMBA (Fig. 9). This study provides evidence, for the first time in ruminants, that MCT1 has a direct role in the transepithelial transport and efflux of the SCFA across stratum spinosum and stratum basale of the forestomach as well as across the basolateral membranes of the large intestine toward the

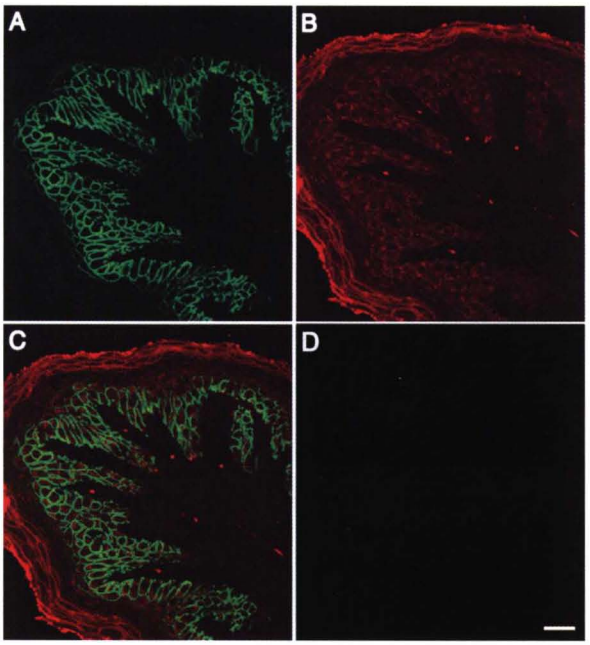

Figure 5. Co-localization of MCT1 and CD147 in the caprine forestomach (represented by rumen). The sections were incubated with a mixture of chicken anti-rat MCT1antibody (diluted 1: 200) and rabbit anti-human CD147 antibody (diluted 1: 10; Abcam, Cambridge, UK) in a humidified chamber at 4°C overnight. For detection of MCT1, the sections were incubated with the diluted (1: 50) donkey anti-chicken IgG conjugated to fluorescein isothiocyanate (FITC) (AP194F; Chemicon International Inc., CA, USA) for 30 min at room temperature; while for detection of CD147, the sections were incubated with the diluted (1: 50) Alexa Fluor-594-labelled donkey anti-rabbit IgG (A21207; Molecular probes Inc., Invitrogen) for 30 min at room temperature. The Immunofluorescent view showing MCT1 (green; A) and CD147 (red; B) in ruminal epithelium. (C): Overlay of MCT1 and CD147 in ruminal epithelium. (D): Negative control. Scale bar = 30 μm. [J. Physiol. (London.), 2006, 576: 635-647]

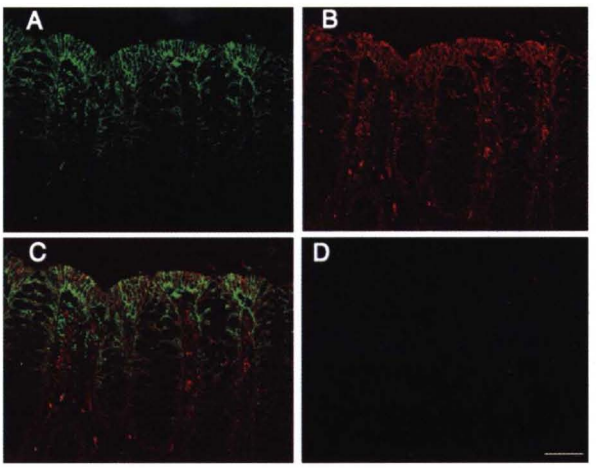

Figure 6. Co-localization of MCT1 and CD147 in the caprine large intestine (represented by cecum). Immunofloresecent view showing MCT1 (green; A) and CD147 (red; B) in cecal epithelium. (C) : Overlay of MCT1 and CD147 in cecal epithelium. (D): Negative control. Scale bar = 30 μ m. [J. Physiol. (London.), 2006, 576: 635-647]

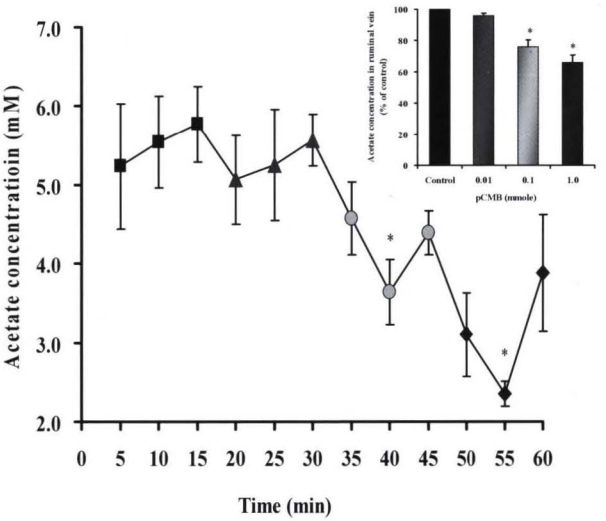

Figure 7. Effect of MCT1 inhibitor; *p*-chloromercuribenzoate (pCMB) on acetate concentration in ruminal vein *in vivo*.
(▲): 0.01 mmole pCMB; (●): 0.1 mmole pCMB; (◆): 1 mmole pCMB were injected over 1 min via a catheter inserted into the right ruminal artery in three consecutive steps. For the control (■), injections were carried out using the same volume of saline. Blood samples were collected from the right ruminal vein at 5 min intervals. Reduction percentage (relative to control) in acetate concentration (inset) induced by injection of different amounts of pCMB (Pooled data over 15 min for each amount). All data points are means ± SE, represented by vertical bars, from four goats. Significant difference was identified at $P < 0.05$ (*). [J. Physiol. (London.), 2006, 576: 635-647]

blood side and it could play a vital role in the intracellular pH regulation.

5. Conclusion

Our studies described the expression, regional distribution and precise cellular localization of MCT1 along the entire gastrointestinal tract of ruminants, and we provided evidence, for the first time in ruminants, that MCT1 has a direct role in the transepithelial transport and efflux of the SCFA and their metabolites across stratum spinosum and stratum basale of the forestomach

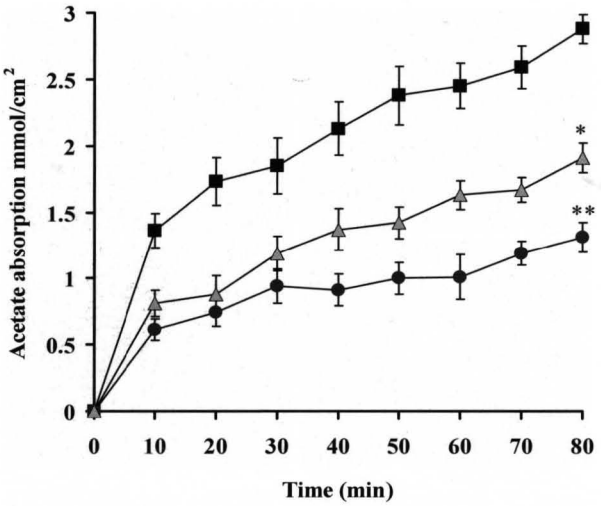

Figure 8. Effect of MCT1 inhibitor, *p*-chloromercuribenzoic acid (pCMBA) on transport of acetate across the caprine ruminal epithelia *in vitro*.
Caprine ruminal mucosae were mounted in Ussing chambers; Acetate (50 mM) was applied into the mucosal side for each of the control (■) and experiment chambers. pCMBA (1 mM) was applied either to the mucosal side (▲) or serosal side (●). All data points are means ± SE, represented by vertical bars. Significant difference was identified at $P < 0.05$ (*) and $P < 0.01$ (**). [J. Physiol. (London.), 2006, 576: 635-647]

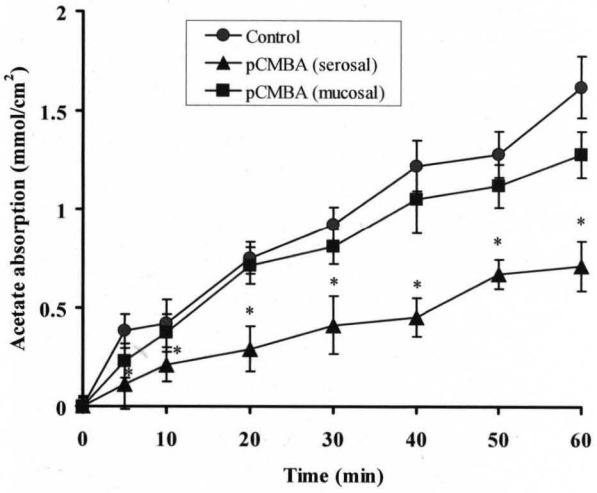

Figure 9. Effect of MCT1 inhibitor, *p*-chloromercuribenzoic acid (pCMBA) on the transport of acetate across the bovine cecal epithelia *in vitro*.
Isolated cecal epithelia were mounted in Ussing chambers. Acetate (50 mM) was applied into the mucosal side for each of the control and experiment chambers. pCMBA (1 mM) was applied either to the mucosal side or the serosal side. All data points are means ± SE, represented by vertical bars, for nine cows. Significant difference was identified at $P < 0.01$ (*). [Exp. Physiol., 2006, 91 : 835-844]

as well as across the basolateral membranes of the large intestine toward the blood side, and hence it could play a vital role in the intracellular pH regulation; additionally, we verified the fact that CD147 is required for the proper localization of MCT1 in the ruminant gastrointestinal epithelial cells.

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