

Use of the Ussing chamber technique to study nutrient transport by epithelial tissues

Liuqin He¹, Yulong Yin^{1,2}, Tiejun Li¹, Rulin Huang¹, Mingyong Xie², Zhenlong Wu³, Guoyao Wu^{3,4}

¹Chinese Academy of Science, Institute of Subtropical Agriculture, Research Center for Healthy Breeding Livestock and Poultry, Hunan Engineering and Research Center for Animal and Poultry Science, Key Laboratory of Agroecology in Subtropical Region, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central China, Ministry of Agriculture, Changsha 410125, Hunan, Peoples R China, ²State Key Laboratory of Food Science and Technology, College of Life Science and Food Engineering, Nanchang University, Nanchang, Jiangxi 330047, China, ³State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100193; and ⁴Department of Animal Science, Texas A and M University, College Station, TX, USA 77843-2471

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1. ABSTRACT

The Ussing chamber provides a physiologically relevant system for measuring the transport of ions, nutrients, and drugs across various epithelial tissues. This article outlines the design, structure, principle, and operation of the Ussing chamber, its application in the field of gastrointestinal barrier function and nutrient transport research, as well as its advantages and limitations. This review serves as a practical guide for investigators who are new to the Ussing chamber and should help researchers better understand this valuable method for measuring the transport of electrolytes, organic nutrients, water, and drugs across the small intestine, placenta, and other epithelial tissues.

2. INTRODUCTION

In 1951, the Danish scholar Hans Ussing invented a device named the Ussing chamber to determine vectorial ion transport through the skin, which has been used for diverse purposes to study the integrity of cell layers and the invasive properties of cancer cells since then (1-4). The Ussing chamber provides a physiologically relevant system for measuring the transport of ions, nutrients, and drugs across various epithelial tissues. One of the most studied epithelial tissues is the intestine, which has been used in several landmark discoveries regarding the mechanisms of ion transport (5-7). Furthermore, the simplicity of the Ussing chamber makes it an attractive *in vitro* model system for studying drug transport (6, 8).

Nutrient transport by epithelial tissues

Today, the Ussing

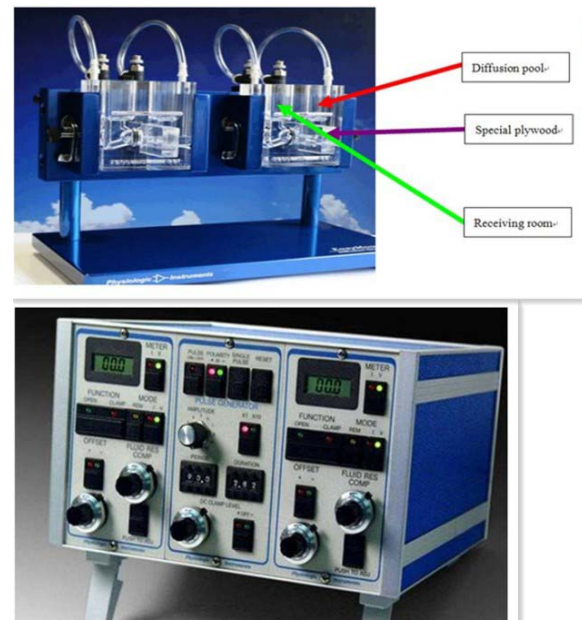


Figure 1. The structure of a circulating Ussing chamber. The picture shows the structure of the chamber and the electrical system of the Ussing chambers which can measure voltage, current, and a voltage/current clamp across an epithelial tissue.



Figure 2. The picture of Ussing chamber manufactured by Physiologic Instrument Inc. Inset: “slider” with pins and aperture for mounting a porcine intestine.

chamber method has been applied to virtually every epithelial tissue in the animal body, including the reproductive tract, exocrine/endocrine ducts, intestine, airway, eye, and choroid plexus (5). This method has also been extensively used in studies of cultured epithelial cells (5-6).

The Ussing chamber is now mainly used in the pharmaceutical field, where microelectrodes are used to detect current changes in intestinal cell membrane ion channels, and in studies of the absorption, permeability and transport of drugs in the

intestine. However, since the design of the Ussing chamber has been continuously improved, it is now also used in many other areas, especially in the field of nutrition. In this article, we review the basic principles of the Ussing chamber technique and its common applications to study physiology and nutrition. In addition, we will address some of the problems and limitations associated with this method.

3. THE USSING CHAMBER SYSTEM

3.1. Basic structure of the Ussing chamber system

The Ussing chamber system has several components, including perfusion, cells, a circuit system, a data collection system and a software support system (4, 7). The Ussing chamber consists of two functional halves, i.e., the chamber itself and the electrical circuitry. The chamber has different sizes and shapes. The electronic circuitry enables the measurement of not only resistance, current and voltage but also complex parameters including impedance and capacitance. The apparatus typically has 2, 4, 6 or 8 perfusion chambers, and either a circulating chamber or a continuously perfused chamber. Figure 1 represents the structure of a circulating Ussing chamber. The circulating chamber includes a U-shaped tube and two compartments, between which is a removable plug-in that can hold a chimerical tissue sample. The continuously perfused chamber contains 2 storage devices; a PE tube provides solution to the two compartments and a valve is used to control the gas flow rate. While the former has been adopted by most laboratories because of its simplicity, the latter offers several distinct advantages (4). For example, during the course of an experiment, substances are usually added to one or both sides of the tube in a sequential manner. It is obvious that once added, the substances remain in the solution until the end of the experiment. Some investigators have overcome this problem by flushing the U-tube during the experiment with fresh solution. However, this can only be achieved after stopping the recording and often results in an altered behavior of the tissue. Because most experiments do not require a control recording after drug treatment, the circulating chamber has proved to be fairly robust and simple to use. The continuously perfused chamber is not as yet commercially available, but can be constructed with the help of qualified machine shops. Figure 2 highlights the features of this design. The two half chambers are designed to minimize the hydrostatic pressure and thus, prevent serious damage to the tissue during perfusion. The circuit system consists of electrodes to measure voltage and current, and a voltage/current clamp across the epithelium. The voltage/current clamp contains a sensitive primary signal acquisition component, a current and fluid impedance compensation scope, a pulse generator for measuring electrical resistance, and a remote interface with an LED display that can be used to control the data-acquisition instruments (4).

3.2. Principle behind the Ussing chamber system

Epithelial tissue consists of a dense array of epithelial cells and the cytoplasm and, in contrast to other tissues, is polar and "tight". Polarity is

generated by the asymmetric distribution of proteins to either the apical or basolateral membranes, which are separated by an assembly of proteins called "tight junctions". The formation and permeability of tight junctions determine the resistance and integrity of the tissue. When Ussing first mounted a sheet of frog skin between two half-cells, he presumably first tried to evaluate its "tightness". Tightness can be expressed in terms of the electrical resistance (R). R is given as $R = \rho \times L/A$ (4, 7-8), where ρ is the specific resistance modulus of the material, L is the length or thickness of the material (constant for each tissue preparation) and A is the area. For a given tissue, R can be broken down into an arrangement of resistors (4-7).

When epithelial tissues transport ions, a transepithelial voltage will be generated, which has been known as the "active transport potential". A basis on the generation of such a transport potential is the asymmetric distribution of ion channels on the apical and basolateral membranes of epithelial cells, which is a prerequisite. Voltage clamp (V_{te}) is known as voltage that the transfer process produces from the transmembrane potential. R_{te} is known as a resistance that exists on the epithelial membrane. Short-circuit current (I_{sc}) is the charge flow per unit time when the tissue is short-circuited, i.e., V_{te} is clamped to 0 mV. Many laboratories prefer to measure the short-circuit current instead of V_{te} because the flow of ions per unit time more accurately reflects the absorptive or secretory capacity of the tissue. In order to measure short circuit current, the epithelium is short circuited by injecting a current that is adjusted by a feed-back amplifier to keep V_{te} at 0 mV (4-9). The amount of current required is adjusted by a feedback circuit and continuously measured. Intermittently, the voltage is clamped to values to 0 mV, thus enabling an estimate of R_{te} . I_{sc} is given by the equation $I_{sc} = V_{te}/R$. From this equation, it is apparent that I_{sc} can be calculated under open circuit conditions when R and V_{te} are known.

In fact, Ussing used this approach, which is generally called "voltage clamping". While this is the accepted technique in most laboratories in studies of transepithelial ion transport, it nevertheless has some caveats. A clamp voltage imposed on the two sides of a living cell, forces electrolytes through the cell which the cell might otherwise not be transporting at that time. Thus, transepithelial ion transport under voltage clamp conditions might not accurately reflect the transport status of that cell. Moreover, cellular responses to movement of the electrolyte might deplete the cell of energy reserves and ultimately damage the tissue. To circumvent these problems, an alternative approach, called "current clamping" has been used. In contrast to a voltage clamp, the tissue in the current clamping method is not exposed to a voltage. Instead, short current pulses are injected via a resistor. At first glance, this is not all that different from a voltage clamp, since in both cases voltage-pulses are applied parallel to the tissue. However, in the case of a current clamp, the current that passes through the tissue creates only a brief voltage deflection, and the cell is left undisturbed for most of the measurement (4-9).

3.3. Operation of the Ussing chamber

A Ussing chamber is used as follows. Once the chambers and solutions are prepared, the system should be flushed with the bath solution without any tissue. If the system is watertight, the temperature should be adjusted to the desired level (37°C). The current and voltage electrodes are then inserted into the half-cells. Once the electrodes are connected to the current/voltage pulse injectors and the volt-/amperometer, respectively, the system should be checked for noise and offset voltages. The latter often occur due to improper storage of the electrodes. Depending on the type of electrode (KCl-filled glass column, Agar-bridge, or Kalomel electrode), small air bubbles can cause the resistance of the electrode to increase, which in turn can cause asymmetries. By turning on the current/voltage pulses, one can estimate the resistance of the empty chambers, which is required for the proper calculation of resistance and currents. Some devices offer the possibility to cancel out both the resistance of the solution and the offset voltage generated by nonequilibrated electrodes. This procedure should be performed before inserting the tissue or filter. The chambers are then disconnected from the supply of solution and the tissue can be mounted. After the system is reassembled, recording can begin. Immediately after tissue insertion, the values of all of the electrical parameters (V_{te} , I_{sc} , R_{te}) tend to oscillate. This is most often caused by the mechanical stress imposed on the sample or residual stimulation. Therefore, the tissue should be allowed to recover for 10 to 30 min before any experimental maneuvers. During that time, continuous recording to document the electrical parameters is necessary. After a stable baseline is reached, the system for data acquisition can be switched to a higher time-resolution and the “real” experiment can begin (4-9, 20).

4. APPLICATION OF THE USSING CHAMBER IN ANIMAL NUTRITION AND PHYSIOLOGY

4.1. Gastrointestinal barrier function

Many researchers consider the Ussing chamber to be the gold standard for determining intestinal barrier function (5, 10-11), which reflects the ability of the gastrointestinal epithelium to protect against invasion by pathogenic antigens. There are several aspects of gastrointestinal barrier function, such as ion secretion, permeability, and mucosal secretion (5-11). Using the Ussing chamber, gastrointestinal barrier function can be studied, with a primary focus on gastrointestinal epithelium permeability, the mechanisms of endotoxin and bacterial movement, and the effects of amino acids [including arginine, glutamine (Gln) and glutamate] and probiotics on gut barrier function.

4.2. Gastrointestinal epithelium permeability using the Ussing chamber

The Ussing chamber system has become an important method for detecting isotopes or fluorescence-labeled macromolecular material in studies of gastrointestinal epithelium permeability, which can be calculated as the proportion of materials that pass through the gastrointestinal epithelium (11). Jutfelt and co-workers (12) used Atlantic salmon to study

gastrointestinal epithelium permeability. The hydrophilic marker ^{14}C -labeled mannitol was used to assess paracellular permeability. In this study, the apparent permeability of mannitol was calculated according to Eq. (1), where dQ/dt is the steady-state appearance rate of radioactivity in the serosal compartment, A is the area of the epithelium exposed, and C_0 is the concentration of ^{14}C -labeled mannitol in the experimental solution in the mucosal chamber (12).

$$P_{app} = dQ/dt \times 1/(A \times C_0) \quad (\text{Eq. 1})$$

To determine the effects of ethanol on intestinal permeability, Ferrier *et al.* (13) treated tissue with ^{51}Cr -ethylenediaminetetraacetic acid (EDTA) and measured radioactivity with a gamma counter, so that intestinal permeability was expressed as a percentage of the total radioactivity administered. Yang and co-workers (14) used Ussing chambers to investigate the effects of total parenteral nutrition on intestinal ion transport and intestinal epithelial permeability, and to assess the role of interferon- γ in the total parenteral nutrition-induced loss of epithelial barrier function (14). Epithelial barrier function was assessed by measuring transepithelial resistance and the transmural passage of ^{51}Cr -EDTA and ^3H -mannitol. Some of their results suggested that total parenteral nutrition significantly affected the intestinal epithelial physiology, stimulated ion secretion and reduced epithelial barrier function, and interferon- γ appears to play an important role in the loss of epithelial barrier function that is associated with total parenteral nutrition. Neirinckx and co-workers (15) showed that the apparent permeability coefficients of turkey and dog jejunum were low and highly variable due to tissue fragility caused by differences in the thickness of the remaining intestinal layers after stripping. Pig and horse jejunum were markedly more suitable for permeability determinations, and only mild signs of deterioration were noted after 120 min of incubation (15). These results suggest that the Ussing chamber technique appears to allow for studies of permeability measurements in animals.

4.3. Studies of intestinal bacterial endotoxin and bacterial replacement with the Ussing chamber

Bacterial endotoxin (named lipopolysaccharide, LPS) plays a central role in triggering the inflammatory cascade that leads to the systemic inflammatory response syndrome (16-20). However, how the LPS that crosses the intestinal barrier to reach the body is still unknown. Benoit and co-workers (21) designed a study to determine whether pure endotoxin could pass across injured rat ileal mucosa in the Ussing chamber. Sprague-Dawley rats were subjected to mild or severe hemorrhagic shock following carotid artery cannulation, and then resuscitated. Control animals underwent only carotid artery cannulation. Bacterial translocation to the mesenteric lymph nodes, liver, or spleen was measured after 24h. Transmucosal passage of fluorescein isothiocyanate (FITC)-labeled *E. coli* C-25, or FITC-conjugated LPS was measured in the Ussing chamber. Severe hemorrhagic shock resulted in a 60%

mortality rate and a 100% incidence of bacterial translocation in surviving animals. Sham-shock rats had a 100% survival rate and only a 33% incidence of bacterial translocation. Transmucosal passage of FITC-E. coli C-25 was similar in both groups; however, the passage of FITC-LPS was not detected. A histological analysis confirmed mucosal injury in the intestinal epithelium of rats that were subjected to severe hemorrhagic shock, and confocal laser microscopy demonstrated the passage of FITC-E. coli C-25, but not FITC-LPS, across the ileal membranes. Therefore, they concluded that pure LPS does not pass across the intestinal mucosa *in vitro* and the transmucosal passage of LPS *in vivo* may be due to the release of bacterial cell wall fragments containing LPS from killed bacteria (21). In an *ex vivo* experiment, Isenmann and co-workers (22) used an Ussing chamber to examine whether aggregation substance (AS), a bacterial adhesin and virulence factor of *Enterococcus faecalis*, promotes bacterial translocation and colonic mucosal invasion. Bacterial translocation through the gastrointestinal tract is a crucial step in the pathogenesis of intra-abdominal infections (22). In this study, they did not use isotope- or FITC-labeled bacteria, but could still obtain satisfactory results, therefore offering a new approach for future research.

4.4. Regulation of gastrointestinal epithelium barrier function

Glutamine has been shown to improve intestinal barrier function and immune function in both *in vivo* and *in vitro* models (6, 23). Gln is an essential nutrient for intestinal mucosa cell metabolism, and plays a very important role in maintaining the structural integrity of the intestinal epithelial surface (13, 23). Julia and co-workers (24) sought to determine the effect of dietary supplementation with Gln on intestinal barrier function and intestinal cytokines in a model of *Escherichia coli* infection. These researchers assigned 21-d-old piglets ($n = 20$) to receive nutritionally complete isonitrogenous diets with or without Gln (4.4 %, w/w) for 2 weeks. Intestinal loops were isolated from anesthetized pigs and inoculated with either saline or one of two *E. coli* (K88AC or K88 wild-type)-containing solutions. Animals that received a diet supplemented with Gln had a decreased potential difference (PD) and short-circuit current (Isc) in *E. coli*-inoculated intestinal loops (PD=0.628 mV; Isc =13.0 $\mu\text{A}/\text{cm}^2$) compared with control-fed animals (PD =1.36 mV; Isc =22.4 $\mu\text{A}/\text{cm}^2$). Intestinal tissue from the control, but not Gln-supplemented, animals responded to *E. coli* with a significant increase in mucosal cytokine mRNA (IL-1 β , IL-6, transforming growth factor- β and IL-10). Tight-junction protein expression (claudin-1 and occludin) was reduced upon exposure to *E. coli* in control-fed animals, but was unaffected in Gln-supplemented piglets. Therefore, supplementation with Gln may be useful for reducing the severity of weaning-related gastrointestinal infections, by reducing the mucosal cytokine response and altering intestinal barrier function (24). L-glutamine is the primary metabolic fuel for enterocytes (25). Furthermore, glutamine is the principal energy source for intestinal enterocytes and is considered to be essential for gut metabolism, structure and function (23). In one experiment, jejunum from rats that had been starved for 48 h were

mounted in Ussing chambers. Glutamine was added to Krebs-buffer at 0.6 mM, 3 mM, 6 mM and 30 mM on the mucosal side. Cr-EDTA permeation, ATP content of the epithelium mucosa and electrophysiology were studied during 180 min of incubation in Ussing chambers. When 30 mM Gln was added to the mucosal side, there was an increase in ^{51}Cr -EDTA permeability ($P < 0.001$). Glutamine did not affect transepithelial resistance, but higher concentrations of glutamine (>3 mM) significantly increased the short-circuit current. The authors concluded that the addition of glutamine at a concentration of 30 mM to the mucosal side in the Ussing chamber led to an increase in ion pump activity and an increase in paracellular permeability (23). Schroeder and co-workers (26) used Ussing chambers to study whether the addition of probiotics (*Saccharomyces boulardii*; a kind of pathogenic yeast) to animal feed affects pig jejunum barrier function. They found that, after the animals were fed probiotics for 8 days, epithelial Isc in the jejunum decreased by 26%, but returned to normal after Day 16. The results indicate that the secretory function of mucosal epithelial cells in the pig jejunum in response to the consumption of *Saccharomyces boulardii* is dependent on the duration of exposure to the probiotic (26).

4.5. Studies on material transport across a gastrointestinal tract using the Ussing chamber

Ussing chambers have been used extensively to study nutrient absorption across gut epithelial tissues for a range of different animal species, including rats and pigs. Ussing chambers have been used to study the uptake of glucose, heparin, oligonucleotides, antibiotics and amino acids (7, 20, 27-41). The Ussing chamber is an *ex vivo* technique in which gut tissue is collected and mounted between two buffer-containing reservoirs (luminal and serosal chambers), thus permitting the study of the absorption of compounds across mounted tissues (28-44).

The Ussing chamber offers a quick and effective method for studying the efficiency of the gastrointestinal transport of different materials, especially glucose, amino acids and minerals. There are published studies involving the use of Ussing chambers to determine whether epidermal growth factor (EGF) affects glucose transport in the jejunum (29). Similar work has been conducted for measuring amino acid transport in the gastrointestinal epithelium using Ussing chambers (30-31). The small intestine can readily take up all amino acids from its lumen via specific carriers (32-33).

4.6. Uses of the Ussing chamber in other fields

Fortuna and co-workers (36) sought to characterize passive transport in the mouse jejunum and the possible active efflux mediated by P-glycoprotein of a series of materials, which comprise some AEDs and metabolites. Absorptive (M-S) and secretive (S-M) transport were analyzed with and without verapamil, which is a widely recognized P-gp inhibitor. The apparent permeability coefficients (P_{app}) in both directions and in the absence or presence of verapamil were determined for each test compound. The results suggested that differences in the bio-disposition of *S*-Lic and *R*-Lic might result from their

distinct interaction with P-gp (36). In that study, the Ussing chamber was useful for predicting F_a of AEDs and for showing that efflux transport, particularly of P-gp, played an important role in absorption (36). Isabelle and co-workers evaluated the feasibility of using an adapter-modified Ussing chamber to assess intestinal transport in colonic biopsies obtained endoscopically from cats and dogs. Fifteen colonic biopsies from four cats and 13 colonic biopsies from four dogs were transferred to a modified Ussing chamber and sequentially exposed to several compounds. Maartje and co-workers (37) found that the *ex vivo* Ussing chamber is a valid alternative for *in vivo* studies, and may serve as a suitable screening tool for studying the effects of nutritional compounds on the release of satiety hormones.

The Ussing chamber can be used to study epithelial tissue, which is widely found throughout the body, such as in various capsules, tubes and inner surfaces of cavities. Epithelial tissues have been reported to have protective, secretory, absorptive and discharge functions, and different epithelial tissues from different regions may have different functions. Additionally, Ussing chambers can be employed to determine the transport of water, minerals, glucose, fructose, amino acids, fatty acids, and vitamins by the placenta of mammals, including the human, pig and rat. Thus, the Ussing chamber system should be useful for further studies in many different fields, such as nutrition, reproduction, toxicology, and pharmacology.

5. STRENGTHS AND WEAKNESSES OF THE METHOD

The principle advantage of the Ussing chamber technique is that it provides a short-term organ culture method that enables the precise measurement of electrical and transport parameters of intact, polarized intestinal epithelium (4-5, 9-10, 20, 40-53). Discoveries made from studies of gene expression in various cell culture models require verification in a physiological context. The weakness of the Ussing chamber technique lies in the fact that it leads to the interpretation of a relatively small number of measurements to describe the complex physiological system of the intestinal mucosa (5, 33, 45-53). This is because the intestinal mucosa contains many types of cells that communicate through a variety of systems, e.g., cell-to-cell contact and paracrine humoral agents, which may not be discerned or even considered during Ussing chamber measurements. Isabelle and co-workers (38) used adapter-modified Ussing chambers to assess endoscopically-obtained intestinal biopsy samples in humans. However, they observed a large differences in their results, which suggests that the clinical use of this method is limited. Another concern that is often raised with the Ussing chamber method is related to the limited viability and optimal function of an *ex vivo* intestinal preparation. Thus, further validation of this method with low-permeability compounds and actively transported compounds is needed. The proper application of experience with the technique and strict adherence to the basic principles of the Ussing chamber method are valuable. However, many of the technical difficulties associated with the Ussing chamber

methodology have been overcome by the introduction of modular systems that make the technique more widely accessible.

6. SUMMARY AND PERSPECTIVES

An Ussing chamber is a scientific tool used to measure both the short-circuit current as an indicator of net ion transport across an epithelium and transport of nutrients by the tissue. Studies of intestinal mucosa with Ussing chambers have provided important findings that have advanced our understanding of transepithelial transport processes. Studies on gastrointestinal barrier function and nutrient transport in animals should help us to better understand the physiology and biochemistry of nutrient utilization. With regard to the use of the Ussing chamber system, the experimental conditions should be as close as possible to those of the gastrointestinal tract and research methods should be improved to make the results more relevant to the *in vivo* situations. The advantages of the Ussing chamber method and advances in the imaging of intestinal epithelial function will greatly advance our knowledge of nutrient transport by epithelial tissues. One example is protein nutrition in animals. Particularly, there is growing interest in the physiological function and nutrition of so-called "nutritionally nonessential amino acids" and their metabolites (54-60). However, quantitative data on their transport by epithelial tissues, such as the small intestine and placenta are lacking. Likewise, little is known about interactions between intestinal microbes and epithelial cells in amino acid metabolism (61-65). This necessary information, in combination with metabolic, cellular and molecular studies (66-80), will provide a much needed basis for defining optimal dietary requirements of all amino acids by animals and humans.

7. ACKNOWLEDGMENTS

This research was jointly supported by grants from the National Basic Research Program of China (2009CB118806 and 2013CB127301), Open Project Program of State Key Laboratory of Food Science and Technology, Nanchang University (SKLF-TS-201108 and SKLF-KF-201216), NSFC (31272463, 3120181331101730, 31110103909, 30901040, 30901041, 30928018, 31172217, 31272450 and 31272451), Chinese Academy of Sciences and Knowledge Innovation Project (XBJ-2011-016), National Fund of Agricultural Science and Technology outcome application (2006GB24910468), Hunan Provincial Natural Science Foundation of China (11JJ4018), Chinese Universities Scientific Funds (No. 2012RC024), the Thousand-People Talent program at China Agricultural University, and Texas A&M AgriLife Research (H-8200).

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Abbreviations: EGF, epidermal growth factor; R, electrical resistance; Gln, glutamine; P_{app} , apparent permeability coefficients; LPS, bacterial endotoxin; FITC, fluorescein isothiocyanate; I_{sc}, short-circuit current; V_{te}, voltage clamp ; R_{te}, transepithelial electrical resistance

Key Words: Ussing chamber, Intestinal, Nutrient transport, Review, Pigs, Reivew

Send correspondence to: Yulong Yin. Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, Hunan, China 410125, Tel: 86-731-84619703, Fax: 86-731-84612685, E-mail: yinyulong@isa.ac.cn