A short-circuit current experiment on epithelial ion transport is described that is suitable for student classes in human and animal physiology. Segments of late distal colon from either pig or cow are obtained from the slaughterhouse depending on the animals' daily schedule. Initial tissue preparation already in the slaughterhouse, cold storage, and proper choice of bath solutions are essential prerequisites for success. Students monitor spontaneous transepithelial voltage and short-circuit current ($I_o$) by use of manually operated voltage clamp units. Two main transport mechanisms are studied, electrogenic Na$^+$ absorption and Cl$^-$ secretion. Electrogenic Na$^+$ absorption is studied by measuring the $I_o$ drop after amiloride. Then Cl$^-$ secretion is stimulated by theophylline and subsequently inhibited by furosemide. In some experiments K$^+$ secretion can be detected by the blocking effect of mucosal Ba$^{2+}$. Response of tissues from pig and cow is qualitatively similar but quantitatively different. The equipment is sturdy and inexpensive, can be provided by most departmental workshops, and has been tested for 3 yr in regular lab courses. Observations made during these experiments are closely related to clinical states, such as secretory diarrhea, cystic fibrosis, and hyperaldosteronism, as well as to the mechanisms of clinically used diuretics.

Understanding of the mechanisms of epithelial transport is one of the major achievements of physiological research during the last few decades. The detection of identical or similar transport systems in epithelia of different organs, the classification of epithelia according to their leakiness, and the revelation of comparable segmental heterogeneity in epithelia of tubular configuration permit a unified treatment of formerly separated chapters of physiology. Examples are absorption and secretion in kidneys and gastrointestinal tract; exocrine secretion in liver, pancreas, and salivary and sweat glands; and fluid balance in the eye, the cerebrospinal fluid system, and the lungs (1, 10). It is therefore desirable to add "epithelial transport" to the list of main topics of basic medical, veterinary, and zoological education. Fortunately, the short-circuit current experiment introduced 40 years ago by Ussing and Zerahn (19) provides an excellent opportunity to train students in epithelial transport physiology (13). In addition to being an introduction into basic electrophysiology, this experiment covers several aspects of humoral and pharmacological regulations of epithelial organ functions, including some pathological states. Also, the issue of keeping organs alive in vitro, and therefore the aspect of organ conservation, is involved.
Although the Ussing experiment has been recognized by physiologists for a long time, only a few physiology and zoology departments have introduced it into their lab courses. In most cases when they did the frog skin was selected and the effect of antidiuretic hormone (ADH; identical to vasopressin) on amiloride-blockable Na⁺ absorption was demonstrated. The frog epithelium survives well under the varying conditions of a student lab course; however, it has sometimes been difficult to motivate the students to do the "frog skin experiment" because human skin does not perform significant net ion and water transport, and electrogenic Na⁺ absorption in other human epithelia is controlled by aldosterone rather than by ADH. The use of aldosterone in mammalian epithelia has been precluded so far for two reasons: first, the full action of aldosterone takes 4–8 h and, second, in mammalian epithelia the apical Na⁺ channel usually deteriorates after 1 or 2 h of in vitro experimentation.

General progress with in vitro experimental techniques now provides the possibility of carrying out prolonged transport experiments in mammalian intestinal mucosa as well. For example, in Ussing experiments on rat distal colon, we have demonstrated that electrogenic Na⁺ absorption in response to aldosterone added in vitro in physiological concentrations could be preserved for 8 h (11). Using these methods, we developed a Ussing experiment on mammalian intestine obtained from the slaughterhouse that we introduced into our lab course in 1989. This experiment proved both technically and didactically successful and has since been adopted by other departments for their lab courses.

METHODS

Experimental Setup

Two students at a time cooperate in performing one experiment by use of the apparatus displayed in detail in Figs. 1 and 2. Eighteen such working places are operated in parallel in three rooms by 36 students.

The setup constitutes a manually operated voltage clamp unit (Fig. 1). All components were manufactured according to our specifications by the departmental machine and electronic shop. A similar Ussing chamber and automatic voltage clamp are manufactured (e.g., model USS15 Ussing system, model DVC-1000 voltage/current clamp for 2 chambers by WP Instruments). In contrast to such an automatic voltage-clamp device, students obtain with our setup a direct experience of "clamping" because they have to operate a simple manual clamp as part of the feedback loop.

Preparation of the Tissue

In the morning of the experiment, a piece of bovine or porcine large intestine is obtained from the local slaughterhouse, where animals have been butchered ~20 min earlier. For this lab course the terminal 10–15 cm of the large intestine, corresponding to the human rectum, are used. The large intestine contains thick muscle layers. Once cut from blood supply, the epithelium relies on diffusion, and therefore the subepithelial tissues have to be removed readily. This is done in an adjoining room of the slaughterhouse by a member of the physiology staff. By use of sharp scissors, the muscle layers are split longitudinally and carefully separated from the mucosa. Next, the remaining mucosal tube is cut open and rinsed with ice-cold trans
Mounting Into Ussing Chamber

In the classroom, the bottle containing the tissues is briefly gassed again and then stored in the refrigerator. Immediately before experiments start (i.e., 2 or 7 h, respectively, after receipt of the tissues from the slaughterhouse), the mucosal preparation is cut into 18 pieces of ~2 × 2 cm (when maximally stretched). Two students perform one experiment, consisting of the following steps:

1) Control of apparatus. Ussing half-chambers are assembled without epithelium, and Tyrode solution is circulated. For 10 min the asymmetry voltage ($V_{as}$) between serosal and mucosal voltage electrodes is monitored and finally adjusted to zero by use of the bias control of the voltmeter. If $V_{as}$ is >5 mV or inconstant, one or both voltage electrodes are replaced. During this period the preparation described under step 2 is carried out.

2) Mounting of epithelia into Ussing chambers. Petri dishes filled with silicone rubber are provided as preparation pads. First they are covered with a conventional transparent plastic foil. Then a specimen is fixed under maximal mechanical stretch by use of five to seven needles, mucosa up, on top of the foil. Next, the serosal half-chamber armed with five pins is pressed firmly into the stretched specimen until the pins penetrate mucosa and foil. The fixing needles are removed, and the whole preparation is lifted from the pad by keeping the foil firmly against the half-chamber. Finally the foil is cautiously removed with the mucosa left on the pins, and the chamber is closed by adding the mucosal half-chamber.

TABLE 1. Bath solutions

<table>
<thead>
<tr>
<th>Electrolytes</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>β-OH butyrate</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>l-glutamine</td>
</tr>
<tr>
<td>KCl</td>
<td>D(+)-mannose</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>D(1)-glucose</td>
</tr>
<tr>
<td>MgCl₂</td>
<td></td>
</tr>
</tbody>
</table>

Solutions are expressed in mmol/l. "Transport" and "storage" solutions contain electrolytes only. "Tyrode" solution used during experiments contains both electrolytes and substrates. Osmolality of transport solution is 216 mosmol/kgH₂O, of Tyrode 267 mosmol/kgH₂O. Transport and storage solutions are equilibrated in the cold state with 95% O₂-5% CO₂ and kept on ice. Tyrode is gassed by the same gas mixture in Ussing chambers by means of bubble lift.
**TABLE 2.**

<table>
<thead>
<tr>
<th>Drug Solution</th>
<th>Volume Added (ml)</th>
<th>Final Concen (mol/l)</th>
<th>Side Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiloride</td>
<td>$10^{-5}$</td>
<td>1</td>
<td>Mucosal</td>
</tr>
<tr>
<td>BaCl$_2$</td>
<td>$5 \times 10^{-2}$</td>
<td>1</td>
<td>Mucosal</td>
</tr>
<tr>
<td>Theophylline</td>
<td>$2 \times 10^{-2}$</td>
<td>5</td>
<td>Mucosal and serosal</td>
</tr>
<tr>
<td>Furosemide</td>
<td>$3 \times 10^{-2}$</td>
<td>1</td>
<td>Serosal</td>
</tr>
<tr>
<td>Nitric acid</td>
<td>6.5%</td>
<td>0.1</td>
<td>Mucosal</td>
</tr>
</tbody>
</table>

All drug solutions except furosemide were prepared by adding drug to ready made Tyrode solution (Table 1). Furosemide solution was taken directly from 25-ml ampuls for clinical use.

The mounting procedure takes altogether < 3 min. This method permits us to mount the mucosa under maximal stretch, which has proved to be important for satisfying results of the experiments. After the chambers are mounted in the holders, the bubble lift (5% CO$_2$-95% O$_2$) is turned on, and the chamber is filled simultaneously on both sides with 10 ml of Tyrode solution (Table 1). The bath temperature of 37°C is maintained by means of a water jacket around the bubble lifts.

**Experimental Protocol**

1) **Control period.** During the first 15 min after filling of the chambers, three measurements of spontaneous open-circuit voltage ($V_{ms}$, mV) and of short-circuit current ($I_{sc}$, μA) are taken (~ 1 every 5 min). In addition, one measurement of tissue resistance ($R_t$, Ω·cm$^2$) is carried out by imposing a voltage step of $ΔV = 5$ mV and reading the necessary clamp current step $ΔI_{cl}$. $R_t$ is then calculated from $R_t = 5$ mV/$ΔI_{cl}$. The determination of $R_t$ permits a control for damaged preparations. If $R_t$ is < 50 Ω·cm$^2$, a new tissue is mounted.

2) **Inhibition of Na$^+$ absorption by amiloride.** This drug is added to the mucosal bath by exchange of 1 ml of mucosal solution for 1 ml of amiloride solution. All drug solutions are listed in Table 2. An immediate shift of mucosal $V_{ms}$ into a more positive direction is taken as an indicator for the presence of electrogenic Na$^+$ absorption. $V_{ms}$ and $I_{sc}$ are measured once immediately before and twice after addition of the drug.

3) **Stimulation of Cl$^-$ secretion by theophylline.** Five milliliters of Tyrode solution on either side are exchanged for theophylline solution, and $V_{ms}$ and $I_{sc}$ are monitored by six readings within ~ 30 min. During this period $V_{ms}$ and $I_{sc}$ increase to more mucosa-negative values because of a strong stimulation of electrogenic Cl$^-$ secretion.

4) **Inhibition of Cl$^-$ secretion by furosemide.** One milliliter of Tyrode solution (still containing amiloride on the mucosal side and theophylline on both sides) is exchanged on the serosal side for furosemide solution. A 20-min follow-up period then occurs with four recordings of $V_{ms}$ and $I_{sc}$.

5) **Inhibition of electrogenic K$^+$ secretion by Ba$^{2+}$.** Some tissues polarize mucosa positive with respect to the serosa, either in the beginning or after mucosal amiloride. This voltage "inversion" (compared with the more frequent state) is caused by electrogenic K$^+$ secretion overruling electrogenic Na$^+$ absorption. Mucosal addition of BaCl$_2$ eliminates K$^+$ secretion and the potential therefore moves in the negative direction.

6) **Optional step: observation of tight junction cation selectivity by diffusion potential.** Five milliliters of the mucosal bath are exchanged twice for 267 mmol/l glucose solution (containing no electrolytes). Thus a NaCl serosa-to-mucosa gradient is established that because of the cation selectivity of the tight junctions creates a diffusion potential, rendering the mucosa more positive with respect to the serosa. This cation selectivity is caused by negative fixed charges within the tight junction. Subsequent acidification of the mucosal bath by addition of 100 μl of 6.5% nitric acid neutralizes the negative fixed charges, causing a reversal of the diffusion potential.
I N N O V A T I O N S  A N D  I D E A S

RESULTS

Figure 3 shows two typical recordings obtained during afternoon lab course experiments. It can be seen that, even after >7 h of storage in cold transport solution, significant short-circuit currents are obtained. Also, typical responses to specific transport inhibitors and to theophylline can be demonstrated. Because $R_\text{t}$ did not vary much during the experiment, voltage recordings (not shown) run nearly parallel to $I_{sc}$ recordings. Between the species there were quantitative differences only: bovine tissues exhibited greater amiloride-sensitive $I_{sc}$ than porcine tissues; their response to theophylline and furosemide, however, was less. Mean effects of single substances, as shown in Fig. 3, are compiled in Table 3. Mean $I_{sc}$ values appeared slightly lower in the afternoon, but this difference was below significance.

DISCUSSION

Mechanisms of Na\(^+\) Absorption and Cl\(^-\) Secretion

In this lab course experiment, students gain practical knowledge about two basic transport mechanisms that are of general importance throughout the organism: electrogenic Na\(^+\) absorption and electrogenic Cl\(^-\) secretion.

In contrast to the cellular mechanisms (see below), the localization of these two pathways within the distal colon and rectum has been clarified only recently. A voltage-scanning technique showed that electrogenic Na\(^+\) absorption is localized in the surface epithelium but not in crypts (15). However, in contrast to previously encountered concepts, electrogenic Cl\(^-\) secretion is localized in both crypts and surface epithelium (14).

Electrogenic Na\(^+\) absorption consists of two transport steps (Fig. 4A). Luminal Na\(^+\) is taken up by a selective channel in the apical membrane. This
Main electrogenic transport mechanisms of mammalian late distal colon as depicted in lab course handout. A: electrogenic Na⁺ absorption; B: Cl⁻ secretion. For explanation see text.
innovations and ideas

channel is induced within 2–8 h by nanomolar aldosterone and can be blocked by $10^{-4}$ mol/l amiloride (11). On the basolateral side, Na⁺ is extruded by the 3Na⁺-2K⁺-ATPase, which is stimulated by aldosterone at chronic exposure and can be blocked by ouabain. Although the basolateral efflux is ATP consuming and directed against an electrochemical gradient, Na⁺ absorption is "primary active" because metabolic energy is directly used for this transport pathway.

Electrogenic Na⁺ transport is the dominating "fine regulator" of the salt and water balance of the body and is present in the distal segments of many excretory pathways, such as distal nephron, distal colon, and excretory ducts of salivary and sweat glands. For example, in rat late distal colon, aldosterone in a range of $0.1-5$ nM regulates electrogenic Na⁺ absorption between zero and high values of $9.4$ kmol·l⁻¹·h⁻¹·cm⁻², or 254 μA/cm², respectively (11). Under stimulated conditions, electrogenic Na⁺ absorption can take place against large concentration gradients, e.g., 60:1 in rat distal colon (9). The experiment described here offers the opportunity to discuss states of hyper- and hypoaldosteronism. With respect to the process of butchering, possible influences of stress on aldosterone liberation and therefore differences in prestimulation of individual samples can be addressed. The rapid and highly selective action of amiloride demonstrates the specific effect of this modern K⁺-saving diuretic. When students have observed in particular its action on $V_{ms}$ also its K⁺-sparing mechanism can be discussed.

Cl⁻ secretion also consists of two transport steps (Fig. 4B). Cl⁻ is taken up from the blood side by a Na⁺-2Cl⁻-K⁺ cotransporter against a slight electrochemical gradient. Cl⁻ secretion is "secondary active" because the cotransporter is driven by the Na⁺ gradient maintained by the primary-active Na⁺-K⁺-ATPase. The Na⁺-2Cl⁻-K⁺ symporter can be blocked by so-called loop diuretics such as furosemide and bumetanide. On the apical side, Cl⁻ leaves the cell through a selective channel, which is opened by cAMP and also by cGMP and Ca²⁺ as second messengers. Thus, Cl⁻ secretion is physiologically controlled by the apical channel. It has been characterized recently as a very-low-conductance (~1-pS) nonrectifying channel (8). Other Cl⁻ channels, especially the outwardly rectifying depolarization-induced ~35-pS channel (ORDIC), but also the ~10-pS cystic fibrosis transmembrane conductance regulator, are probably not involved in cAMP-induced net Cl⁻ secretion. In the thick ascending limb of Henle's loop of the kidney, Cl⁻ is not absorbed because of mirror-like arrangement of these transporters. Thus the loop diuretics act here from the luminal side.

Transepithelial electrogenic Cl⁻ secretion is the basic mechanism that drives active salt and fluid excretion by the gastrointestinal tract and the exocrine glands. This transport pathway exists likewise in stomach, small intestine, and large intestine. In the experiment described here Cl⁻ secretion is stimulated by a very high concentration of theophylline. Although theophylline is known to have several side effects, the use of this drug for stimulation of secretion is standard even in scientific experiments (2, 5, 7, 12, 14, 16). For a lab course it is especially advantageous because it is the least expensive drug. The topic of stimulated and impaired Cl⁻ secretion is treated in accompanying seminars in context with the pathological states of secretory diarrhea and cystic fibrosis. The use of the "loop" diuretic furosemide permits a discussion of mechanism and side effects (K⁺ waste) of this highly potent diuretic. Diuretics like amiloride or furosemide would act in principle in all target epithelia. Their specificity is given by quantitative reasons only: clinical dosage is too small to become effective except in the kidney, where both diuretics are concentrated during tubular passage (10).

Didactic main issues of the tutorials accompanying these experiments are membrane transport mechanisms, basic electrophysiology, mechanisms of action of specific transport inhibitors such as diuretics, hormonal regulation of transepithelial net transport, and the pathophysiological significance of impairment or dysregulation of epithelial transport.

Technical Aspects of the Experiment

The experiment described here has proven to be sufficiently simple and sturdy to be technically suitable even for experimentally untrained stu-
dents. We have used it for three years, and it has been adopted by the Departments of Veterinary Physiology and Animal Physiology here at Free University, Berlin, as well as by the Department of Human Physiology of Humboldt University, Berlin. The setup is not very expensive if the equipment is produced by the university workshop. With proper supervision the error rate is small. As far as individual experiments fail, most difficulties occur with air bubbles or breaks in the agar bridges, damaged epithelia, contaminated chambers, bad electrodes, or use of wrong solutions. Sometimes voltages and currents are small and require careful reading down to 0.1-mV accuracy. Nevertheless, ~9 of 10 students are able to reproduce the drug effects displayed in Fig. 3.

If in a particular experiment there is no reasonable short-circuit current, we let the student switch to the optional tight junction experiment. It works even in “bad” tissues, because it does not depend on active transport. Here the cation selectivity of the tight junction is studied, which is provided at physiological pH by negative fixed charges within the tight junctions (17). These fixed charges are “titrated” to positive by mucosal addition of any inorganic acid that lowers the pH of the mucosal bath (we used nitric acid). This causes the polarity of the diffusion potential to turn, indicating that the tight junction has become anion selective. This little experiment provides an example of extracellular tight junction regulation. Many other substances or conditions alter tight junction permeability by intracellular mechanisms mediated by the cytoskeleton (for review see Ref. 18).

We recommend the Ussing experiment for students in their second rather than first year of physiology training. A full appreciation of the multiple clinical aspects of epithelial transport requires sufficient knowledge of the general functions of epithelial organs.

Comparison With Other Data From Pig and Cow

Electrolyte transport in mammalian colon and rectum has been studied mainly in small laboratory animals, such as rat, rabbit, and guinea pig, but the same transport functions have been identified also in human preparations. Thus, besides its more general aspects, the experiment also teaches human intestinal physiology. Several comprehensive reviews on large intestinal transport are available (e.g., 3, 6, 12). So far, little data are available from pig and cow. Our results in pig distal colon as reported here compare well with published data from the literature. In stripped pig distal colon, Breves et al. (4) obtained values of about the same size in the control state. They also observed a tendency of $I_{sc}$ to decrease under $10^{-4}$ amiloride, which, within the limits of errors, is compatible with the data reported here. A strong stimulating effect on $I_{sc}$ of the 20 mM theophylline was seen in partially stripped descending pig colon by Argenzio and Whipp (2). They did not test for furosemide or bumetanide, however, and concluded from concentration measurements of $\text{Na}^+$, $\text{K}^+$, $\text{Cl}^-$, and $\text{PCO}_2$ that $\Delta I_{sc}$ after theophylline was mainly due to electrogenic $\text{HCO}_3^-$ secretion. Our finding that the stimulated $I_{sc}$ was partly inhabitable by furosemide supports a considerable contribution of electrogenic chloride secretion. Because we use more distal segments than Argenzio and Whipp, differences may be explained by segmental heterogeneity. In contrast, in distal colon of guinea pigs it was shown by Clauss et al. (5) that piretanide, another loop diuretic, had a definite inhibiting effect even on spontaneous $I_{sc}$. In these animals, amiloride ($10^{-4}$ M) and $\text{Ba}^{2+}$ ($10^{-2}$ M) also had effects similar to those reported here for pig distal colon.

With respect to bovine colon or rectum, the literature provides very little comparable data. Only recently, McKie et al. (16) compared electrolyte and fluid transport in descending colon of cow and sheep in Ussing chambers. Again, the exact localization of their preparations along the large intestine is not described precisely, leaving segmental differences open to speculation. The very low resistance of only 16 $\Omega \cdot \text{cm}^2$ suggests that their samples may have been mounted without, or with very little, stretch. Because theophylline ($10^{-2}$ M) induced a reduction of net $\text{Na}^+$ absorption similar to that in Argenzio’s experiments on pig distal colon, the segments in these two studies are probably comparable.

Our data indicate that, in pig and cow late distal colon, the pattern of transport systems is qualita-
tively similar. We conclude that these tissues provide useful models for teaching basic epithelial transport physiology with reference to the human body.

Preliminary test experiments on a variety of preparations from the slaughterhouse were carried out with the dedicated help of volunteering medical students. We also gratefully acknowledge the skilled technical assistance of Ursula Lempart, Sieglinde Luderitz, and Detlef Sorgenfrei. We thank the staff of the Institut für Fleischhygiene, Außenstelle Schlachthof, Freie Universität Berlin, for their cooperation.


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References


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