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TRANSMURAL ELECTRICAL POTENTIAL ACROSS RAT JEJUNUM. EFFECTS OF EVERSION AND RAPID TEMPERATURE CHANGES

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SUMMARY

Immediately after warming everted sacs of rat jejunum from 0° to 37°, the transmural potential difference (PD) averages 5.1 mV, a value close to that which can be recorded *in vivo*; however, the PD is not maintained at this level but rapidly declines to a relatively steady value of about 1.4 mV. This rapid decline is apparently not caused by depletion of energy supplies and is not seen unless the intestine is everted. The non-everted sac maintains a PD similar in magnitude to that which can be recorded *in vivo* for a much longer time than does the everted sac, and may be a better preparation for certain types of study *in vitro*.

INTRODUCTION

When everted rat jejunum was incubated *in vitro*, using a medium with normal Na^+ concentration but without organic substrate, a transmural electrical potential difference (PD) averaging 1.5 mV, serosal side positive, was recorded by BARRY *et al.*[1]. However, when these authors made similar measurements from rat jejunum *in vivo*, a considerably higher PD (mean = 4.7 mV) was recorded[1]. LYON AND CRANE[2] obtained somewhat larger values (mean = 3.20 mV) with everted sacs of rat jejunum, but still not as large as have been recorded *in vivo* (ref. 3; R. D. BAKER, unpublished observations). This discrepancy is also true for rat duodenum[4,5]. Since the transmural PD is thought to depend principally upon mucosal to serosal Na^+ transport by the epithelial cells[2], it would seem that normal rates of Na^+ transport are not maintained by the everted small intestine studied *in vitro*.

In preliminary experiments we noticed that when recording was started soon after beginning incubation at 37°, the PD was fairly high (3–4 mV) but was not maintained. It decreased during the first few minutes to a relatively steady value of about 1.5 mV. BARRY *et al.*[1] have previously made this same observation. It seemed that the initial PD immediately after warming to 37° might represent more closely the PD *in vivo*. The present report is concerned with this initial PD and with its subsequent decline.

Abbreviation: PD, potential difference.

METHODS

Male Holtzman rats weighing between 190 and 360 g were used. Each rat was fasted for 18–24 h, and then anesthetized with ether; its small intestine was rinsed with ice-cold Krebs–Ringer bicarbonate solution[6] and removed by cutting at the radix. After cutting away the mesentery, a 5-cm segment of mid-jejunum was everted and tied to a glass cannula. The technique was essentially that of CRANE AND WILSON[7] as modified by LYON AND CRANE[2] for PD measurements. The serosal solution was 0.5 ml Krebs–Ringer bicarbonate buffer and the mucosal solution was 8.0 ml Krebs–Ringer bicarbonate buffer (pH 7.4). The mucosal solution was gassed with 5 % CO_2 in O_2. Polyethylene tubes filled with 3 M KCl in 3 % agar led from mucosal and serosal solutions to calomel electrodes. Potentials were recorded on a Grass polygraph with a Model 5P1 d.c. preamplifier. The input impedance was $1.0 \cdot 10^6\,\Omega$. This was judged adequate since identical PD's were obtained using a Keithley model 610B electrometer with an input impedance of $10^{14}\,\Omega$. After balancing the PD caused by electrode asymmetry, the system was calibrated through the source from the 5P1 preamplifier.

The jejunal segment was kept immersed in Krebs–Ringer bicarbonate buffer at 0° from the moment of excision until the KCl bridges were inserted; this usually took about 9 or 10 min. We recorded at 0° for 1–2 min, and then quickly transferred the cannula, with attached intestine, KCl bridges, and gassing tube to 8.0 ml of mucosal solution at 37° while recording continued. In some experiments a small thermistor (Cole-Parmer 8456) was placed in the serosal solution; temperature was recorded on the polygraph from a Yellow Springs Model 43TD Tele-Thermometer.

RESULTS AND DISCUSSION

A record obtained in a typical experiment is shown in Fig. 1. The PD was 0.5 mV at 0°. While the serosal temperature increased to 37° the PD increased, in two phases, to 4.2 mV. The PD then gradually declined to a relatively steady value of 1.5 mV even though the temperature remained at 37°. This response to warming was consistently observed. Mean PD values for 16 rats are shown in Table I. The maximum

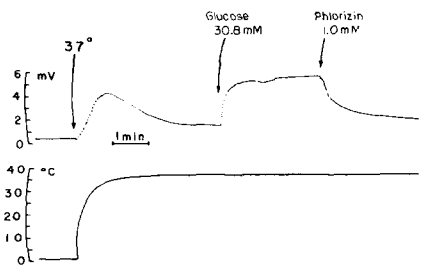


Fig. 1. Transmural PD across everted rat jejunum —effect of warming from 0° to 37°. At the first arrow the intestine was quickly transferred to a new mucosal solution at 37°. Transfer took no longer than 5 sec. 4 min later, 1.0 ml of 5 % glucose solution in Krebs–Ringer bicarbonate buffer was added to the mucosal solution to give the final concentration indicated in the figure. Phlorizin solution (1.0 ml) was added at the last arrow to give the final concentration indicated. The curved lines used for the ordinate scales indicate the curvature of the lines on the chart paper which have not been reproduced here.

TABLE I

TRANSMURAL PD* AT 0° AND AT 3 TIMES AFTER WARMING TO 37°

	0°	After warming to 37°		
		Peak	4 min	5 min
Everted	0.6 ± 0.08	5.1 ± 0.4	1.6 ± 0.1	1.4 ± 0.1
Non-everted	0.7 ± 0.09	6.3 ± 0.4	5.5 ± 0.4	5.8 ± 0.4

* Each value is the mean PD (in mV) ± S.E. for 16 animals in each group.

PD, obtained within 1 min after transfer to 37°, was similar in magnitude to that recorded *in vivo*. After 5 min at 37° the subsequent decline in PD was very slow. The usual effects of D-glucose and phlorizin in the mucosal solution[1,8–10] are also shown in Fig. 1. D-Galactose had the same effect as D-glucose.

The hump in the PD curve following warming was not blocked by 10^{-4} M phlorizin in both mucosal and serosal solutions, although, of course, the response to actively transported sugars was prevented. Neither was the hump consistently affected by 10^{-4} M ouabain in the serosal solution. Curiously, we also could not detect any consistent effect of ouabain on the steady potential or on the glucose-induced potential, a result which is inconsistent with previous observations on rat jejunum[11,12]. Four different sources of ouabain were employed: Sigma, Calbiochem, Nutritional Biochemicals, and Lilly. Only the last preparation, which was from the same manufacturer's lot as that used by LYON AND CRANE[11] (I. LYON, personal communication) and for which we thank Dr. J. M. MCQUIRE of the Lilly Research Laboratories, resulted in occasional inhibition of the PD, especially noticeable when the jejunum was not everted. The other preparations either had no effect or caused a small transient increase in PD. Digitoxin at a concentration of 10^{-4} M in the serosal solution also failed to inhibit the PD. Insensitivity of transport processes in rat small intestine to cardiac glycosides has been reported previously[13–15].

The possibility that this transient elevation in PD is an artifact caused perhaps by asynchronous warming of the KCl bridges has been ruled out by testing dead intestine (boiled for 10 sec) and rat diaphragm, neither of which produced any PD upon warming. However, everted rabbit gall bladder, also an asymmetric epithelial membrane with low resting PD, showed a response to warming similar to that of everted rat jejunum.

The rise in PD with warming would be expected from the effect of temperature on the Na^+ pump and on energy supply. But the reason for the rapid drop in PD while the temperature remained constant is not obvious. A ready explanation is that the gut runs low on fuel. But the PD can be immediately restored by adding an actively transported sugar to the mucosal solution even if the sugar is not metabolizable. Furthermore, if the decline in PD is caused by depletion of fuel supplies, the cycle should not be repeatable. Fig. 2 shows that when the intestine was transferred back to 0° for 2 min and then returned to 37°, a second response was obtained. A third response is also shown in Fig. 2. In six experiments done according to the routine shown in Fig. 2, the first, second and third peaks averaged 6.2, 2.2, and 1.6 mV, respectively. The duration of exposure to 0° between 1 min and 15 min had no

appreciable influence on the magnitude of the second response. If the second response were caused by recuperation of energy supplies at 0° (which seems unlikely), or by accumulation of Na^+ in the epithelial cells at 0°, the second response should be dependent on duration of exposure to the cold solution. We conclude that sufficient

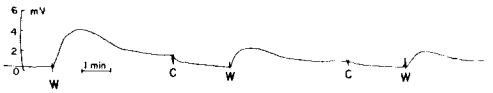
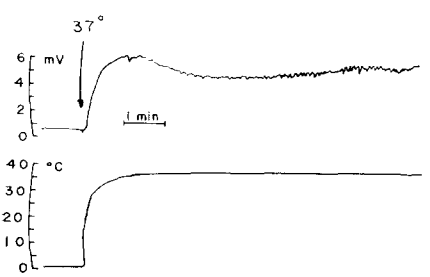


Fig. 2. Transmural PD across everted rat jejunum —effect of repeated warming and cooling. The segment was quickly transferred to a warm (37°) mucosal solution at W and transferred to a cold (0°) mucosal solution at C. An artifact appears in the record at the moment of each transfer as the outer electrode loses contact with mucosal solution.

fuel is available at 37° to produce a PD well above that which is actually maintained. Second and third responses could not be obtained if the mucosal solution were gassed with 5 % CO_2 in N_2 instead of O_2 throughout the experiment or if 10^{-3} M 2,4-dinitrophenol was present in the incubation medium. Therefore, the response to warming was dependent upon oxidative metabolism. These metabolic inhibitors had little or no effect as long as the intestine was maintained at 0°, and the first response to warming took place even in the presence of anoxia or dinitrophenol.

If the failure of everted gut to maintain a normal PD is not caused by depletion of fuel supply, it might be caused by absence of glucose at the transport site in the brush borders, glucose which might normally arrive at that site in a retrograde fashion from the vascular system. However, BARRY *et al.*[1] showed that a very high concentration was necessary for glucose in the serosal solution to increase the PD appreciably, and this effect was blocked by mucosal phlorizin. Since phlorizin does not decrease the PD recorded *in vivo*[1], we conclude that maintenance of a higher PD *in vivo* than *in vitro* is not due to endogenous glucose.

The work of CHALFIN, COOPERSTEIN AND HOGBEN[16], showing bullfrog intestine *in vitro* to be more permeable when everted than when not everted, suggested that eversion *per se* rather than removal from the animal may lead to failure of the PD. Consequently, experiments were run without everting the jejunal segment. Typical results are shown in Figs. 3 and 4, and average PD values for 16 rats are shown in Table I. During warming the PD increased in two phases to a maximum which averaged 6.3 mV. It then often decreased slightly but usually recovered so that it



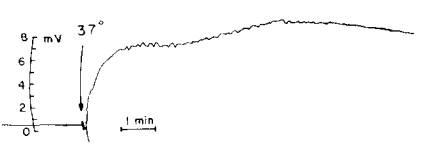


Fig. 3. Transmural PD across non-everted rat jejunum—effect of warming from 0° to 37°.

Fig. 4. Transmural PD across non-everted rat jejunum —effect of warming from 0° to 37°. This example illustrates the two-phase rise in PD during warming better than the example in Fig. 3 and also shows the second peak sometimes observed.

sometimes reached a second peak at about 5 min after warming. The PD was significantly higher than with everted jejunum at the peak and especially at 4 and 5 min after warming, and resembled, much more closely, the normal PD found *in vivo*. The PD then slowly declined, but even after 30 min it was still 3 or 4 times greater than with everted gut. When the non-everted intestine was cooled back down to 0°, the PD dropped to under 1 mV, but with subsequent rewarming to 37° the PD was completely restored to its previous value. TIDBALL *et al.*[17] have found the non-everted rat intestinal sac to utilize O_2 almost as rapidly as the everted sac; apparently the mucosa still receives as much O_2 even though the mucosal solution is not directly oxygenated. The marked difference in PD between everted and non-everted jejunal segments was confirmed using a technique in which the inner solution was continuously circulated and oxygenated.

When non-everted segments were used, ripples always appeared in the PD record after the serosal solution reached 37° (Figs. 3 and 4). These ripples were synchronous with intestinal contractions and could be stopped promptly by adding ouabain (10^{-4} M) to the serosal solution.

When segments were everted, but then immediately turned back to their normal orientation, the PD was maintained at values nearly as high as those found with non-everted segments. Therefore, the failure of everted gut to maintain a normal PD is not mainly the result of trauma during the eversion process, but is associated with the state of being everted.

As stated above, bullfrog intestine is more permeable when everted than when not everted[16]. TIDBALL AND CASSIDY have confirmed this observation* and have extended it to rat small intestine[17] in which the equivalent pore radius was found to be enlarged when the gut was everted*. These observations suggest an explanation for the failure of everted intestine to maintain a normal PD. Perhaps the permeability to ions, and, therefore, the transmural electrical conductivity, increases after everted intestine is warmed to 37°. An increase in transmural conductivity would tend to dissipate the PD by internal short circuiting. Experiments are now in progress to measure short circuit current and transmural conductivity. These results will be reported later, but it is already apparent from these studies that transmural conductivity of everted jejunum does not increase while the PD is failing, but remains relatively constant. So another explanation must be found.

Perhaps the simplest hypothesis is that, when everted jejunum is warmed, the permeability of the epithelial brush borders to Na^+ is decreased. Consequently the intracellular Na^+ concentration diminishes as the Na^+ pump, located at the basal and lateral borders of the epithelial cells, extrudes Na^+. As intracellular Na^+ concentration decreases, the pump slows with a decline in PD. The brush border of non-everted jejunum, either *in vivo* or *in vitro*, according to this hypothesis, has a relatively high permeability to Na^+ so that the pump is continually supplied with adequate Na^+ to maintain a high PD. Other possible explanations, involving transport of other ions, *e.g.*, Cl^-, could be formulated.

In conclusion, the everted rat jejunum seems to develop changes in ion transport during relatively brief maintenance at 37°. The non-everted intestine may, for some purposes, be a better preparation *in vitro*.

* C. S. TIDBALL AND M. M. CASSIDY, personal communication.

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