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RESPONSE OF TRANSMURAL ELECTRICAL PARAMETERS ACROSS IN VITRO EVERTED SACS OF HAMSTER JEJUNUM TO VARIATIONS IN OXYGENATION RATE

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SUMMARY

1. The response of the electrical potential difference, short circuit current, and resistance across everted sacs of hamster jejunum to variations in the mucosal solution gassing rate was investigated.

2. Contrary to previous reports by others, it was found that the potential difference responds to increases in mucosal solution gassing rate by increasing in magnitude during the first 20 min of incubation.

3. The increases in potential difference were paralleled by increases in short circuit current but not by changes in resistance.

4. Increases in mucosal solution gassing rate increased epithelial cell O_2 availability and this effect was determined to be due to increased stirring by gas bubbles. From the data, it was deduced that the minimum thickness of the mucosally located functional unstirred layer is between 0.08 and 0.16 cm when less than the full magnitude of electrical activity is observed across the everted sac preparation.

5. Serosal N_2 or O_2 had little or no effect on electrical parameters under maximum mucosal oxygenating conditions but dramatically affected these parameters when less than maximum mucosal oxygenating conditions were used.

6. Qualitative variations in the magnitude of the short circuit current across this preparation with respect to Cl^- dependence were demonstrated. These variations were dependent upon the level of O_2 availability, being apparent at high levels of O_2 and absent at low levels.

7. It is concluded that the thickness of mucosally located unstirred layers can determine the O_2 availability to the mucosa of everted sacs of hamster jejunum and thereby influence the observable magnitude and pattern of ionic dependence of the short circuit current across this preparation.

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INTRODUCTION

Even though a large amount of information regarding the transmural and transmembrane electrical activity of the in vitro small intestine has been gained from past studies, much of this information has been based on the assumption that the conditions of incubation were able to provide enough oxygen to meet the demands of the tissue for ion transport. However, recent investigations indicate that this assumption may not be generally true. For example, Munck [1] found that a net chloride secretion accounted for most of the short circuit current (I_{sc}) across the sheet preparation of rat jejunum used in his studies whereas a net sodium absorption accounted for the I_{sc} across the everted sac preparation of this tissue in the studies of Barry et al. [2]. Munck [1] suggested that these differences in ion transport could result if it were more difficult to provide O_2 to the everted sac preparations than to the sheet preparations. A more complex situation with respect to oxygenation was revealed by the observations of Baker et al. [3], confirmed on the sheet preparation by Munck [1], that the presence or absence of mucosal solution O_2 was virtually without effect on the transmural potential difference (PD) and I_{sc} across sheets and sacs of hamster jejunum whereas changes in serosal solution O_2 dramatically affected these electrical parameters.

Taken altogether these studies indicated that the conditions ordinarily used for oxygenating the mucosal solution were unable to support full levels of ion transport by either the everted sac or the sheet preparations, and that the quantitative and perhaps qualitative nature of ion transport was more susceptible to oxygenating conditions than had been considered. Hence, investigations were undertaken in this laboratory to determine whether more vigorous conditions of mucosal solution oxygenation could effect transmural ion transport as indicated by the time course of the I_{sc} and/or the qualitative nature of ion transport as indicated by the time course of transmural electrical parameters in ion-substituted media. In the course of this work, it was found that the method of varying the O_2 availability from the mucosal solution operated through a progressive reduction in the thickness of mucosally located unstirred layers. Hence, information about the minimal thickness of these layers during less than optimal oxygenating conditions could be deduced.

METHODS

8–12-week-old Syrian golden hamsters which were allowed free access to food and water were sacrificed by cervical crushing. Everted sacs of jejunum were prepared from tissue located 3.5–7 inches from the duodenal end of the intestine and were secured to the lower end of the gut tube and serosal current electrode described later. Enough buffer to produce a 1-cm initially serosal-positive hydrostatic head was added to the serosal compartment and the gut tube assembly was placed into the gut tube assembly holder. Recording of electrical parameters was initiated within 4 min from time of sacrifice. While the condition of imposing an initial 1-cm serosally positive hydrostatic head was found to be advantageous in demonstrating an effect of gassing rate on the parameters studies, it was not a prerequisite to such an effect.

Incubation apparatus and electrical measurements

An apparatus similar to that used by Barry et al. [2] was used in these studies. Briefly, the apparatus consists of a mucosal coil and serosal electrode of Ag/AgCl for depolarizing current passage, mucosal and serosal Agar/KCl salt bridges for monitoring transmural PD, a vertical displacement lucite gas dispersion bulb for gassing the mucosal solution, and a tube from which to suspend an everted gut sac. The vertical displacement gas dispersion bulb contained four small holes for gas escape distributed symmetrically about the center of the flat top surface which was located about 2 cm below the lower end of the hanging gut sac. The placement of the gassing bulb in such a manner forced the gas bubbles which escaped into the mucosal solution to rise in close proximity to the mucosal surface of the hanging gut sacs. Gas was introduced into the serosal solution by way of a short length of polyethylene (PE 10) tubing which opened half way down into the solution just above the serosal salt bridge. The rate at which gas entered the mucosal and/or serosal solution was carefully controlled by a series of gang valves and monitored via a Gilmont No. 11 flowmeter (mucosal solution gas flow) or a Gilmont No. 10 flowmeter (serosal solution gas flow).

The gut tube assembly holder was housed in a 50 cm^3 plastic centrifuge tube containing 27 ml of buffer at 37 C. In some studies, the assembly holder was housed in a vertical cylinder containing 105 ml of buffer so that the mucosal solution could be mechanically stirred by use of a Corning PC 351 magnetic stirrer which was operated at stop No. 4. Tight fitting junctions, in addition to alignment marks, insured reproducible geometry of electrodes with respect to the everted gut sacs.

The technique used by Clarkson and Toole [4] of correcting for electrode asymmetries and fluid resistance by plotting current-voltage relationships obtained in the presence and absence of tissue was used to obtain the corrected PD (mV), I_{sc} ($\mu A/cm^2$), and R ($\Omega \cdot cm^2$) in these studies. This process was performed mathematically by an IBM 1130 computer which was programmed to compute the true transmural electrical parameters from the slope and y intercept obtained from computed least squares regression lines of the E-I plots obtained in the presence and absence of the tissue. For the regression lines Y A + BX in the absence and y a + bx in the presence of the tissue, the true I_{sc} across the entire gut sac (a A)/(B b) where a A is the true transmural PD and B b is the total tissue resistance. The equations apply when the direction of current passage is depolarizing in the presence of tissue and in the opposite direction when there is no tissue. The final expression of the electrical parameters reflects the surface area of the gut sacs which was obtained from measurements assuming the sacs to be right circular cyclinders*.

Since the value of the naturally occurring transmural PD was frequently changing during the passage time of a set of current steps, it was necessary to correct the value of the recorded transmural PD at any current step in order to obtain accurate measurement of the I_{sc} and R. This was accomplished by subtracting the zero current PD before current passage from the zero current PD after current passage, dividing this value by the number of current steps, and subtracting the quotient from the recorded PD at each current step. Since the time for the passage

* The gut sacs were approx. 3.5 cm in length and 0.5 cm in width and had a dry weight of 9.5 mg/cm^2.

of a set of current steps rarely exceeded 25 s, the assumption of a linear change in the monitored transmural PD during the time of current passage can be vindicated by inspection of the PD traces.

Krebs-Ringer bicarbonate buffer [5] which had been pre-equilibrated with O_2/CO_2 (95 : 5, v/v) was used in these studies unless noted otherwise*. Chloride-free media were prepared by replacing chloride salts with sulfate salts and adding mannitol to maintain osmolarity [6]. Potassium-free media were prepared by equimolar substitution of choline chloride for KCl and NaH_2PO_4 for KH_2PO_4. The pH of these buffers ranged from 7.15 to 7.3 and unless stated otherwise the gas phase was O_2/CO_2 (95 : 5, v/v). Lactic acid was assayed by the method of Barker and Summerson [7].

Summaries of the statistical comparisons of the values obtained are provided in the legends to the figures. Since most of the statistical comparisons of electrical parameters were highly significant ($P < 0.001$ in most instances), where possible, only the time points of those groups which were not significant from the same time point of the control group at the $P < 0.05$ level are listed. Control groups to which the Student's t-test comparisons were made are indicated.

RESULTS

Fig. 1 shows that the transmural PD across everted sacs of hamster jejunum increases dramatically when the rate of gassing the mucosal solution increases from 5 to 100 cm^3 per min and that this increase is greatest after about 12 min of incubation. Although the literature (Lyon and Crane [8] using rat jejunum) indicates that the magnitude of the transmural PD across intestinal tissue is generally like that

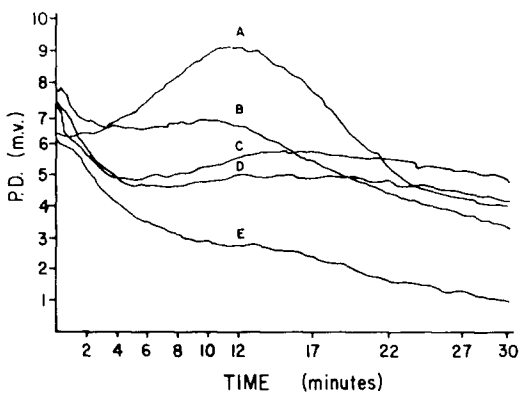

Fig. 1. Preliminary study showing the effect of varying the mucosal solution gassing rate on transmural electrical potential difference (PD) of hamster jejunum bathed on both sides with Krebs-Ringer bicarbonate buffer. Jejunal sacs were mounted in the apparatus described in Methods and gassed at rates indicated with O_2/CO_2 (95 : 5, v/v) administered via PE 50 tubing opening near the bottom of the mucosal compartment. Gassing rates: A = 100 cm^3/min, B = 75 cm^3/min, C = 50 cm^3/min, D = 25 cm^3/min, E = 5 cm^3/min.

* The buffer contained no glucose except in the lactate study in which the concentration was 5 mM.

obtained for the lowest two gassing conditions of this study, Clarkson and Toole [4], demonstrated that the magnitude of the I_{sc}, and presumably the PD, across everted sacs of rat ileum in the presence of 10 mM glucose followed a time course similar to that followed by the PD in the upper curve of Fig. 1. These investigators suggested that low concentrations of Ag^+, released by current passage, potentiated while higher concentrations inhibited the magnitude of the I_{sc} in their preparations. However, since no current was passed in the study of Fig. 1, the observed pattern of increasing PD with increasing mucosal solution gassing rates must reflect an increased availability of oxygen to the intestine.

Upon repeating these studies, a consistent finding worth noting was that the magnitude of PD obtained with any particular gassing rate was much lower if the gas bubbles rose up the side of the incubation tube rather than in close proximity to the gut sac. In view of this, and of the fact that the buffer was pre-equilibrated with the gas phase, the apparent increase in intestinal O_2 availability with increasing gassing rates could not have been due to an increased O_2 content of the buffer or the gas phase above the buffer.

While it is possible that changes in tissue resistance could have accounted for the observed changes in PD, Fig. 2 illustrates that it is the time course and magni-

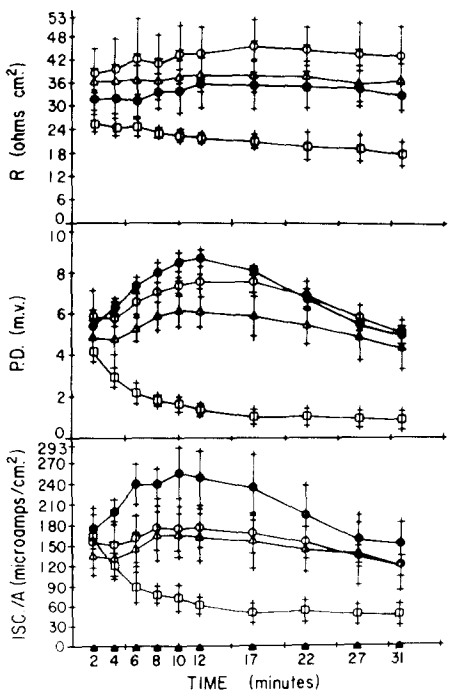

Fig. 2. Influence of varying the mucosal solution gassing rate on electrical parameters across everted sacs of hamster jejunum incubated in Krebs-Ringer bicarbonate buffer. Conditions were as stated in Methods with the mucosal solution gassed at a rate of 5 cm^3/min (□), 25 cm^3/min (△), 50 cm^3/min (○), 75 cm^3/min (●). Time point comparisons which were not significantly different from control values at $P < 0.05$ in Student's t-test were I_{sc}/A at min 2 and 4 of 25- and 50-cm^3/min group, min 2 of 75-cm^3/min group, and PD at min 2 of 25-cm^3/min group. Each point was determined with six gut sacs. The control group is (□). Hash marks in the figure represent S.D.

tude of the I_{sc} and not the R which parallels the PD under similar gassing conditions. Thus, it would appear that ion pumping by the epithelial cells (as indicated by the I_{sc}) is responding to changes in the mucosal solution gassing rate. The fact that the I_{sc} changes in parallel with the PD, indicates that changes in PD may be taken to reflect the level of ion pumping by this tissue as they are predominantly not accounted for by changes in tissue resistance. However, an increase in tissue resistance does result from an increase in gassing rate, as can be seen in Fig. 2. Although this increase does occur, the resistance remains relatively stable throughout the period of observation and in no way parallels the time course or sequence of PD and I_{sc} changes.

The Student's t-test comparisons indicate that the PD, I_{sc}, and R of the 25-, 50-, and 75-cm^3/min groups are significantly greater than that of the 5-cm^3/min group after the second minute of incubation. The choice of control group was predicated on the fact that the curves of PD obtained at this rate are similar in shape and magnitude to those reported in the literature for this tissue [8]. Between group comparisons by the 5% least significant difference test at minute 12 indicates that the transmural PD of all groups differ significantly from one another; that the group mean I_{sc} of the 5- and 75-cm^3/min groups differ significantly from all other groups values but that of the 25- and 50-cm^3/min groups do not differ significantly from one another, and that the group mean resistance of the 25-, 50- and 75-cm^3/min groups are significantly higher than that of the 5-cm^3/min group while the mean resistance of the 75-cm^3/min group is significantly lower than that of the 50-cm^3/min group. This latter difference indicates that the significant difference in PD between the 50- and 75-cm^3/min group can be accounted for entirely by an increase in ion pumping (I_{sc}) by the 75-cm^3/min group while the significant difference in PD between the 25- and 50-cm^3/min group is due to an increase in I_{sc} and R of the 50-cm^3/min group. While the overall inferences suggested by this statistical analysis are difficult to interpret, they indicate that increases in the mucosal solution gassing rate above control levels leads to three effects. These are: (a) a pattern of increasing ionic pumping at increasing rates of gassing, (b) a similar and perhaps related pattern of increasing tissue resistance with increasing gassing rates up to a maximum resistance value, after which further increases in gassing rate result in (c) a significant decrease in tissue resistance from the maximum value. While the latter mentioned effect could indicate a direct "toxic" effect of O_2 on the epithelial cell membranes [9], the fact that the I_{sc} is further stimulated at the highest gassing rate employed in this study is suggestive that no toxic effect was observed on ion pumping. This is consistent with the results of Allen and Rasmussen [10] who demonstrated that high tensions of O_2 resulted in damage to the membranes of red blood cells and frog skin (toxic effect) while cell metabolism was increased rather than decreased.

In order to ascertain that an increased epithelial cell O_2 availability does result from increased rates of mucosal solution gassing, lactate production by everted sacs was measured after 30 min incubation when the mucosal solution gassing rate was either 5 or 75 cm^3/min and the results are shown in Table I. Although the tissue content of lactate was not measured, the finding that the serosal content, serosal to mucosal concentration ratio, and total amount of measured lactic acid was significantly greater ($P < 0.001$, 0.001, 0.01, respectively) for sacs gassed at 5 cm^3/min indicates that increasing rates of mucosal solution gassing do provide increasing amounts of O_2 to the everted gut sacs. Since the epithelial cell is the major site of

TABLE I

CONCENTRATIONS OF MUCOSAL AND SEROSAL LACTATE IN EVERTED SACS OF HAMSTER JEJUNUM WITH VARIATIONS IN MUCOSAL SOLUTION GASSING RATE

Everted jejunal sacs were incubated for 30 min in 27 ml Krebs-Ringer bicarbonate buffer, pH 7.15, containing 5 mM D-glucose in both mucosal and serosal solutions. The mucosal solution was gassed with O_2/CO_2 (95 : 5, v/v) at the specified rates in such a manner that the gas bubbles rose in close proximity to the gut sac. The total serosal amount and concentration and S/M ratios were significantly different at $P < 0.001$ when comparing the two groups. Number of animals appear in parentheses. Values are mean ± S.E., S/M = serosal/mucosal concentration of lactate, lactate assayed by method of Barker and Summerson [7].

Gassing rate	Solution	Total (μg)	Lactate	
			Total mM	S/M
5 cm^3/min (6)	Serosal	196.8 ± 15.0	3.18 ± 0.24	61.39 ± 6.99
	Mucosal	134.8 ± 19.3	0.055 ± 0.007	
	Both	331.8 ± 28.9		
75 cm^3/min (6)	Serosal	64.4 ± 10.0	1.17 ± 0.12	21.13 ± 3.56
	Mucosal	144.6 ± 14.7	0.058 ± 0.005	
	Both	208.9 ± 20.9		

lactate production by this tissue [11] and is the probable site of ion transport that generates the I_{sc} across this tissue [12], these results, coupled with the preceding demonstration of increasing early magnitudes of I_{sc} with increasing mucosal solution gassing rates, indicate that epithelial cell ion transport rates increase in response to increasing epithelial cell O_2 availability.

While the in vitro gassing rate effect is primarily on epithelial cell O_2 availability, the question remains as to why all rates of gassing the mucosal solution are not equally effective in providing O_2 to these cells. Since an increase in perturbation of the liquid adjacent to the mucosal surface of the gut sacs is an expected consequence of providing an increase in the mucosal solution gassing rate, a study was undertaken to determine what effects stirring the mucosal solution had on the transmural electrical parameters. The incubation apparatus modified for stirring was used in these studies. The mucosal solution stirring and gassing procedure is given in the legend to Fig. 3. The results shown in Fig. 3 indicate that after the sixth minute of incubation stirring the mucosal solution results in a significant increase in PD and I_{sc} across everted hamster jejunum. When the same rate of gassing and stirring was used, but the gas bubbles rose up the stirring vortex, and additional increase in PD and I_{sc} was observed and these differences were significant. Thus, it appears that stirring the mucosal solution increases the sensitivity of everted sacs of hamster jejunum to the mucosal solution gassing rate. These observations are consistent with the effect that a mucosally located unstirred layer would be expected [13] to exert on tissue O_2 availability. This effect can be examined mathematically by considering the theoretical example of Kidder [14] which has been modified to account for the fact that only the mucosal solution was gassed in this study.

The diagram in Fig. 4 illustrates the situation of an everted gut sac which receives a supply of O_2 from the mucosal solution only. The assumptions that the serosal solution provides negligible O_2 to the everted jejunum under the specified

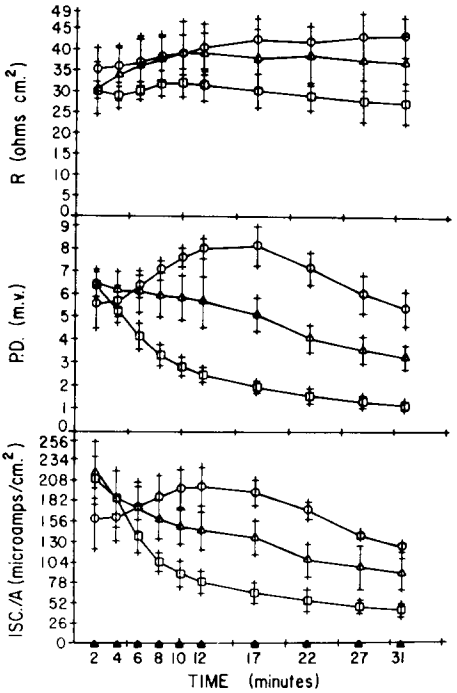

Fig. 3. Study showing the effect of vigorously stirring the mucosal compartment and the additive effect of mucosal solution gassing on electrical properties across everted sacs of hamster jejunum. Conditions are: gassing the upper 1 cm of the mucosal compartment at 10 cm^3/min with O_2/CO_2 (95 : 5, v:v) via PE 50 tubing so that bubbles do not come close to gut sac, unstirred group (□), as above but with magnetic stirring of mucosal solution at stop No. 4 of a Corning PC 351 magnetic stirrer, stirred group (△), stirring as above but with gas bubbles rising from bottom of vortex and coming in close proximity to the gut sac, stirring and vortex gassing group (○). Time point comparisons which were not significantly different from control group values at $P < 0.05$ in Student's t-test were: I_{sc}/A at min 2 and 4 of stirred group and min 4 of stirring and vortex gassing group: PD at min 2 of stirred group and min 2 and 4 of stirring and vortex gassing group; and R of stirred group at 2, 4, 6, and 10 min. Each point was determined with six gut sacs. The control group is (□). Hash marks in the figure represent S.D.

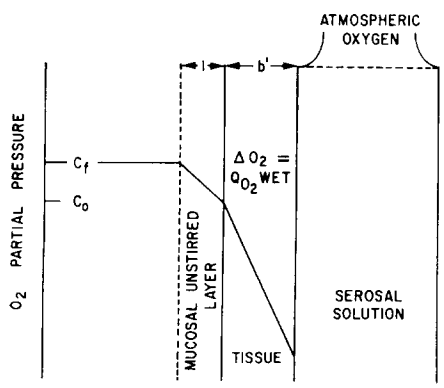

Fig. 4. Diagrammatic representation of a gut sac receiving O_2 from mucosal side only. See text for description.

conditions and that diffusion of O_2 up to the mucosal surface acts as diffusion up to a plane sheet are simplifications which allow an uncomplicated discussion of the situation. When all of the O_2 which gets into the tissue from the mucosal solution is utilized, the flux* of O_2 across the mucosally located functional [15] unstirred layer is given by:

$$J_{O_2} = b' Q_{O_2 wet} \tag{1}$$

where b' is the limiting thickness of respiring tissue which can be supplied with O_2 under the stated conditions and $Q_{O_2 wet}$ is the rate of O_2 consumption per unit volume of tissue and by:

$$J_{O_2} = \frac{d(C_f - C_0)}{l} \tag{2}$$

where d is the coefficient of diffusion of O_2 in free solution, C_f and C_0 are the partial pressures of O_2 in the stirred mucosal solution and the solution adjacent to the mucosal membrane, respectively, and l is the thickness of the functional unstirred layer. Setting Eqn 1 equal to Eqn 2 and solving for C_0 yields:

$$C_0 = C_f - \frac{b' Q_{O_2 wet} \cdot l}{d} \tag{3}$$

Since stirring would be expected to decrease the magnitude of l [16], Eqn 3 shows that stirring should increase the availability of O_2 at the mucosal surface (C_0) of the everted jejunal sacs. Hence, evidence presented thus far appears to constitute a clear experimental demonstration of the effect of a natural unstirred layer on an in vitro intestinal transport parameter which is dependent upon O_2 availability.

From these considerations, it can then be predicted that when the epithelial cells are not adequately supplied with O_2 from the mucosal side of the intestine there will be an effect of serosal gassing on ion transport and conversely when the mucosal O_2 supply is adequate serosal gassing will have no effect. This point was investigated and the results are shown in Fig. 5. The procedure used in varying the O_2 availability from the mucosal solution is given in the legend to the figure. The results indicate that when an additional supply of O_2 is provided from the serosal side of the everted gut sac there is an immediate increase in the transmural PD and I_{sc} when minimal conditions of mucosal solution gassing are used but not when maximum conditions are used. The values of the transmural PD and I_{sc} across everted sacs exposed to minimal conditions were significantly larger ($P < 0.01$) 2 min after serosal 95 % O_2 gassing was initiated, and remained significantly larger than the pre-serosal 95 % O_2 gassing values for the duration of the serosal gassing phase. When maximum conditions of mucosal solution gassing were employed, the transmural PD and I_{sc} continued to decline from their pre-serosal gassing values as was the case when there was no serosal gassing (see Fig. 2). It thus appears that curves of transmural PD and I_{sc} such as the lower and higher curves of Fig. 2, respectively, may represent inadequate and adequate oxygenation of the intestinal epithelium. Curves of PD and I_{sc} obtained under con-

* Where J_{O_2} is in units of ml · cm^{-2} · h^{-1}.

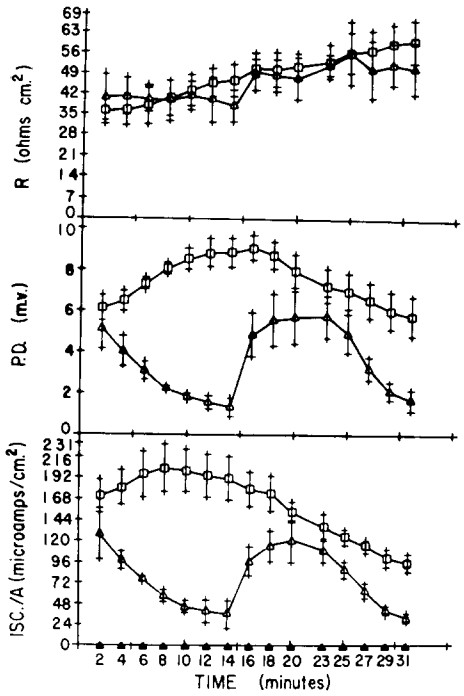

Fig. 5. Effect of serosal O_2 and N_2 on electrical parameters across everted sacs of hamster jejunum incubated in Krebs-Ringer bicarbonate buffer. Mucosal gassing conditions were as follows: maximum conditions: the mucosal solution was gassed with O_2/CO_2 (95 : 5, v/v) at 75 cm^3/min in the presence of magnetic stirring in such a manner that gas bubbles rose up stirring vortex in close proximity to gut sacs (□); minimum conditions: the upper 1 cm of mucosal solution was gassed with O_2/CO_2 (95 : 5, v/v) at 10 cm^3/min in absence of magnetic stirring (△), in both cases 0.2 cm^3/min serosal O_2/CO_2 (95 : 5, v/v) was initiated at 14 min and changed to 0.2 cm^3 N_2/min at 20 min. Each point was determined with six gut sacs. Hash marks in the figure represent S.D. Probability of observing a value of t greater in magnitude than the calculated values is given in table below when electrical parameters of each group at 16, 18, and 20 min, and 23, 25 and 27 min were compared to those values at 14 and 20 min, respectively. n.s., not significant.

Time comparison:		*P* less than value listed					
		16–14	18–14	20–14	23–20	25–20	27–20
RA	□	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	△	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PD	□	n.s.	n.s.	n.s.	n.s.	n.s.	0.02
	△	0.01	0.01	0.01	n.s.	n.s.	0.05
ISC/A	□	n.s.	n.s.	0.01	n.s.	0.002	0.001
	△	0.02	0.01	0.01	n.s.	n.s.	0.02

ditions similar to those for the higher curves of Fig. 5 except with serosal N_2 gassing throughout the duration of the study did not differ from the upper curves of Fig. 5 indicating that serosal N_2 has no effect on electrical parameters when maximum mucosal gassing conditions are employed.

The question of adequate O_2 availability and the unstirred layer

Hill [17] has shown that the limiting thickness (b') of tissue which can be adequately supplied with O_2 from one side is given by:

$$b' = \left(\frac{2DC_0}{Q_{O_2 wet}}\right)^{\frac{1}{2}} \tag{4}$$

where D is the diffusion coefficient of O_2 in the tissue and the other symbols are as stated above. Substituting Eqn 3 for C_0 in Eqn 4 and solving for b' gives the relationship between limiting tissue thickness and unstirred layer thickness as:

$$b' = \left[\frac{2DC_f}{Q_{O_2 wet}} \cdot \left(\frac{Dl}{d}\right)^2\right]^{\frac{1}{3}} = \frac{Dl}{d} \tag{5}$$

Eqn 5 shows that when D, C_f, d, and $Q_{O_2 wet}$ are constant, the maximum thickness of intestine that can be provided with O_2 from one side is dependent upon the thickness of the functional unstirred layer. The relationship between b' and l is plotted in Fig. 6 and was calculated for everted hamster jejunum from Eqn 5 using the following constants at 37 °C: D $1.4 \cdot 10^{-5}$ cm^2/atmosphere per min [17], D $7.5 \cdot 10^{-5}$ cm^2/atmosphere per min [14], C_f 0.95, $Q_{O_2 wet}$ hamster jejunum 0.04 cm^3/cm^3 per min. The value for the $Q_{O_2 wet}$ was calculated from a value of 12 μl/mg dry weight per h given by Wilson and Wiseman [18] for the mid-intestine of hamster by assuming the wet weight to be five times the dry weight [19] and the specific gravity of hamster jejunum to be 1 g/cm^3. The upper horizontal dotted line in Fig. 6 represents the calculated thickness of the mucosa of hamster jejunum (0.0154 cm) which would have to be supplied with O_2 in order to generate the maximum I_{sc} across this tissue assuming the entire mucosa is active in ion pumping. The value for the thickness of hamster jejunal mucosa was obtained by estimating the percent mucosa of the total thickness of hamster jejunum from photographs [19]

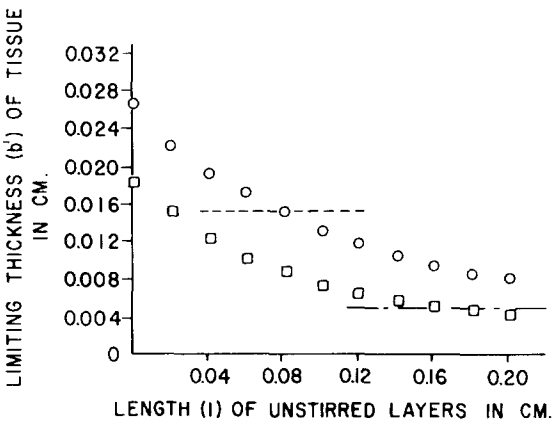

Fig. 6. Relationship between the limiting thickness of tissue which can be supplied with O_2 from one side and the length of a functional unstirred layer located on that side of the tissue. ○, points based on total mucosa $Q_{O_2 wet}$; □, points based on estimated epithelial cell $Q_{O_2 wet}$.

and multiplying by the average thickness of hamster small intestine obtained from values listed by Wilson and Wiseman [18]. As can be seen from Fig. 6, it would take an unstirred layer 0.08 cm thick on the side of the tissue which is being gassed before the entire thickness of the mucosa could not be supplied with adequate O_2. However, the value, 0.08 cm, is based on a flat tissue with no intervillus spaces. The actual thickness of mucosa measured through a villus is several times larger. Since diffusion of O_2 occurs in part by way of the intervillus spaces, neither of the above assumed values are an accurate estimation of the thickness of mucosa which would have to be adequately oxygenated from one side in order to support full ion pumping activity.

The villi of the hamster jejunum are approx. 0.04 cm in length [19] while the maximum thickness of tissue which can be adequately supplied with O_2 from one side when no unstirred layer is present is 0.026 cm as shown in Fig. 6. Thus, oxygen diffusing through the long axis of the villi would not be adequate for cells located at the bottom or top of the villi were the oxygen supplied only from the top or bottom, respectively. Since the diffusion coefficient of O_2 is greater for solutions than for tissue, O_2 could be supplied to the epithelial cells located along the villi by diffusing through the intervillus spaces. If one assumes that a single layer of epithelial cells covering the villi and lying within the crypts of the villi is responsible for generating the I_{sc} across hamster jejunum, then the thickness of tissue which must be supplied with O_2 from the mucosal solution in order to sustain ion pumping can be estimated as being no larger than 0.005 cm, (lower horizontal line in Fig. 6) which is the estimated thickness of the epithelial cells [19]. Since Dickens and Weil-Malherbe [11] have shown that the Q_{O_2} of the mucosa is approx. 1.5 times the Q_{O_2} of the total thickness of rat jejunum, the value for the Q_{O_2} of the epithelial cells of the hamster jejunum can be estimated as being two times higher than the Q_{O_2} of the entire thickness of the jejunum. This value was used in calculating the limiting thickness from Eqn 5 which is shown as the lower plotof Fig. 6 with □ representing the points of calculation. As can be seen from Fig. 6, an unstirred layer 0.16 cm in height from the base of the villi would have to be present on the mucosal surface of the intestine before even the deepest epithelial cells would experience oxygen deprivation and 0.20 (i.e. 0.16+0.04) cm thick before cells located at the tip of the villi experience oxygen deprivation. Hence, when less than full electrical activity of an everted hamster jejunal sac is observed due to inadequate oxygenation conditions, a minimum unstirred layer 0.08–0.16 cm thick can be assumed to exist on the mucosal side of the gut if O_2 is supplied from the mucosal solution regardless of whether or not an additional supply of O_2 is made available from the serosal solution.

Ion replacement studies

Munck [1] proposed that greater O_2 availability to sheets of rat jejunum could explain why the I_{sc} across this preparation was due to 70 % Cl^- secretory and 30 % Na^+-absorptive net flux while the I_{sc} across everted sacs of rat jejunum [2] was due primarily to a Na^+-absorptive flux. Munck's [1] proposal was tested in the following way: Everted sacs of hamster jejunum were incubated in Cl^--free medium with a mucosal gassing rate of either 5 or 75 cm^3/min. The results are shown in Fig. 7. Increased gassing causes increased PD and I_{sc}. However, there is a conspicuous absence of the hump seen in Cl^--containing media (Fig. 2) on the 75

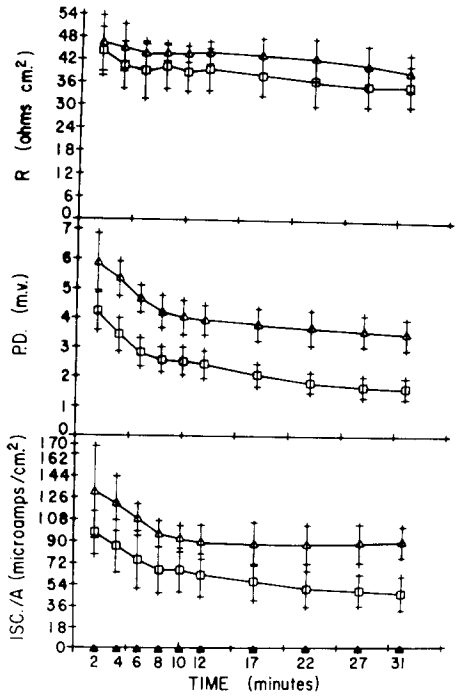

Fig. 7. Effect of variable mucosal solution gassing rates on everted sacs of hamster jejunum incubated in a Cl^--free medium with SO_4^{2-} as the replacement anion and mannitol added to maintain osmolarity. The mucosal solution gassing rates were 5 cm^3/min (□) and 75 cm^3/min (△). Resistance of two groups did not differ significantly at $P = 0.05$. I_{sc}/A not significantly different at min 2; all other points differed significantly. Each point was determined with six gut sacs. The control group is (□). Hash marks in the figure represent S.D.

cm^3/min PD and I_{sc} curves whereas the curves obtained with 5 cm^3/min are similar in shape and magnitude to those obtained in the presence of Cl^-. Assuming the difference between Figs 2 and 7 at the high gassing rate to result from Cl^- flux. these data agree with Munck's [1] proposal.

However, while the results presented in Fig. 7 are suggestive that Cl^- involvement in ion transport at high levels of O_2 availability are responsible for generation of the hump on the I_{sc} curve, additional evidence obtained in K^+-free media indicates that the Cl^- involvement may not be simple. The transmural electrical parameters across everted hamster jejunum incubated in K^+-free media while the mucosal solution was gassed at either 5 or 75 cm^3/min are shown in Fig. 8. Inspection of the 75 cm^3/min PD and I_{sc} curves shows the effect of a reduced hump being formed on the PD curves of some gut sacs. However, in general, it would appear that extracellular K^+ may be involved in the Cl^--dependent ion flux mechanism.

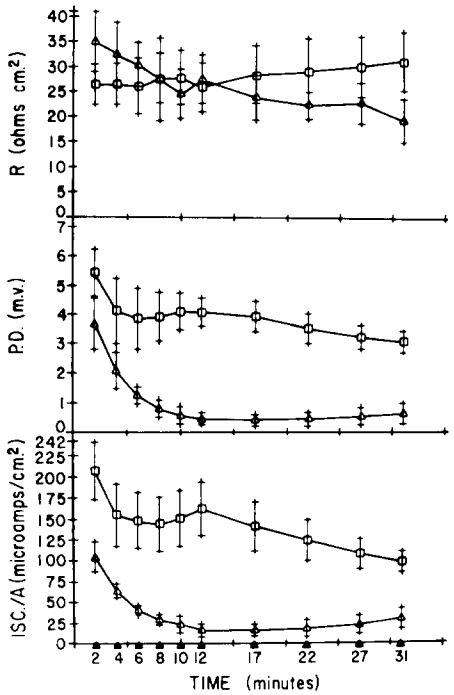

Fig. 8. Effect of variable mucosal solution gassing rates on everted sacs of hamster jejunum incubated in a K^+-free media with choline as the replacement cation. Mucosal solution gassing rates were 5 cm^3/min (△) and 75 cm^3/min (□). Each point was determined with six gut sacs. The control group is (△). Hash marks in the figure represent S.D. All values of two groups differed significantly except *R* at min 4, 6, 8, 10, 12, and 17.

DISCUSSION

While numerous investigations of the electrical parameters across small intestinal tissue have been performed, past studies have generally not indicated that the electrical parameters were being measured on tissue preparations in a state of oxygen deprivation. Although Pietra and Cappelli [20] demonstrated that bicarbonate buffer which had been pre-equilibrated with O_2/CO_2 (95 : 5, v/v) could not saturate the O_2 utilization rate of everted sacs of rat jejunum, they did not attempt to correlate transport function with the apparent availability of O_2.

The present study demonstrates that an electrical parameter (the I_{sc}) which is an indirect [21] but quantitative [12] measure of the active transport of ions by the epithelial cells of this tissue, varies in response to variations in O_2 availability during the first 20 min of incubation. In fact, the magnitude and time course of the I_{sc} across this tissue can be used as a measure of O_2 availability to the epithelial cells. Based on the criteria of magnitude and time course of the I_{sc}, a number of studies in the literature must have been performed on oxygen-deprived tissues and could have yielded results which confused rather than resolved the issue being investigated. The fact that the Cl^- dependence of the I_{sc} in the present studies does not become manifest until higher levels of epithelial cell O_2 availability are reached may be taken as an illustration of the point.

The results of the present electrical studies were obtained in the absence of any actively transportable organic substrates. Thus, the studies shown in Figs 2, 3, and 5 along with the lactate data of Table I demonstrate that: (1) mucosal solution oxygenating conditions do affect the magnitude of the I_{sc} in a predictable manner in that increasing mucosal solution gassing rates results in increasing magnitudes of I_{sc} when no O_2 is provided from the serosal side (Fig. 2); (2) the effect of increasing mucosal solution gassing rate on I_{sc} is due to a stirring effect (Fig. 2 results section and Fig. 3) which increases the epithelial cell O_2 availability (Table I); and (3) that the maximum mucosal solution gassing rate employed is capable of supporting the maximum attainable [21] I_{sc} across this preparation (in the absence of transportable organic substrates) even in the presence of serosal solution anaerobiosis (Fig. 5). These results are consistent with the effect that large mucosally located unstirred layers, which were previously shown to be present on non-villus tissue by Diamond [22], would be expected to exert on O_2 availability. Furthermore, the results suggest that the reason Munck [1] and Baker et al. [3], were unable to demonstrate an appreciable dependance of I_{sc} on mucosal solution O_2 when no organic substrates were present was because their preparations contained an appreciable mucosal diffusion barrier to O_2. Clearly, if there had been no mucosal diffusion barrier in the studies of Munck [1] and Baker et al. [2], O_2 would have reached the I_{sc}-generating epithelia and supported the full magnitude of the I_{sc} irregardless of whether the I_{sc}-generating epithelia were located along the length of the villi or in the crypts as has been suggested. The present study indicates that the diffusion barrier could have been an unstirred layer. Westergaard and Dietschy [23] have shown that in addition to unstirred layers a second complicating factor is that the mucosa swells to such an extent that the intervillus space becomes occluded after 30 min in vivo incubation of rabbit jejunum at 37 C and that such occlusion actually prevents diffusion of test molecules into the intervillus area. Since such tissue swelling is common to the rat and hamster intestine when incubated in vitro and in fact has been shown to be essentially complete after 15–20 min for intestinal tissue from these animals [24], it is likely that it was present in the studies of Munck [1], Baker et al. [3] and the present one. In fact, mucosal swelling could account for the fact that the maximum I_{sc} obtained at the higher gassing rate decreased after the 12th minute of incubation in the present study since this would correspond approximately with the time course of mucosal swelling for this tissue [24]. In the studies of Munck [1], and Baker et al. [3] tissue swelling could have accounted for the lack of effect of mucosal O_2 on I_{sc}. In addition, if the O_2 being provided from the serosal side was sufficient to support the full magnitude of the I_{sc} in the preparations of Munck [1] and Baker et al. [3] one would expect no effect of removal of mucosal O_2 on the I_{sc}. There are, therefore, several alternative explanations for why the results of the present study do not agree with previously published observations. It should be apparent that the data demonstrate quite clearly what effect the presence of mucosally located unstirred layers can be expected to exert on the O_2 availability and I_{sc} of in vitro intestinal preparations.

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