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Effect of Glucose and Diuretics on Intracellular Potentials of Mouse Intestinal Mucosa[1] (39748)

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The intracellular or mucosal potential of intestinal mucosal epithelium has been stuided in various species of animals: rabbit (1), rat (2, 3), tortoise (4), and bullfrog (5). The magnitude of the transmural potential, V_{sm}, varied from 2–11 mV in the various species and that of the intracellular potential, V_{mc}, which was oriented so that the cellular side was electronegative, varied markedly ranging from 6–53 mV. Our laboratory used mouse intestine to study the ion transport and reported that there is an active sodium absorption and chloride secretion across the small intestine (6). It is not known how the intracellular potential of mouse intestine compares with that reported for other species. Recently we have also shown that diuretic agents, ethacrynic acid (EA), furosemide, and amiloride inhibit both Na^+ and Cl^- transport and reduce V_{sm} across the intestinal membrane in the presence of gluocse; such an inhibitory effect of EA on Na^+ and Cl^- transport was insignificant when glucose was not present in the bathing solution (7). We have, therefore, postulated that EA acts mainly on a Na-glucose cotransport-facilitated system at the mucosal side of the epithelium while furosemide and amiloride affect mainly the ionic conductance of the membrane. Chez *et al.*, (8) used the rabbit ileum to study the effects of EA and reported that EA increases the permeability of the brush border membrane and its inhibition on Na^+ is near the serosal membrane. This question has been raised: Can we identify the membrane permeability to Na ion and to the Na-glucose cotransport system by means of the intracellular potential measurement as in the presence and absence of glucose and diuretics? The present investigation gives a positive answer to that question and further substantiates our postulation on the site of action of EA as electrolyte transport across the intestinal mucosa.

Methods. Female albino Swiss-Webster mice, averaging 30–40 g in weight, were kept in a constant-temperature room with food and water *ad libitum* for more than 1 month before being used. They were killed by decapitation. One section of the upper jejunum was cut off and rinsed with mammalian Krebs-Ringer solution. The intestine was then opened along the mesenteric line and mounted in a lucite chamber custom-made for this study, as illustrated in Fig. 1. The mucosal side was facing up and the serosa was down. Both surfaces were bathed with a mammalian Krebs-Ringer solution with or without glucose being added. The bathing solution was continuously stirred by bubbling with 95% O_2:5% CO_2 gas. The chamber was placed inside a Faraday cage on a stone table supported by a vibration-absorbing mechanism.

Two pairs of electrodes were used: one pair of calomel electrodes for PD measurements and one pair of Ag, AgCl electrodes for sending current and measuring the resistance. The appropriate amplifiers were used for impedance matching and the potential difference was recorded on a Varian A25 two-channel recorder. The microelectrodes were prepared from 1- to 2-mm diameter Pyrex glass tubing by a microelectrode puller. The diameter of the microelectrode tip averaged 0.5 μm. The microelectrodes were then filled under vacuum, first with methanol, then water, and finally with a 3 *M* KCl solution. The tip

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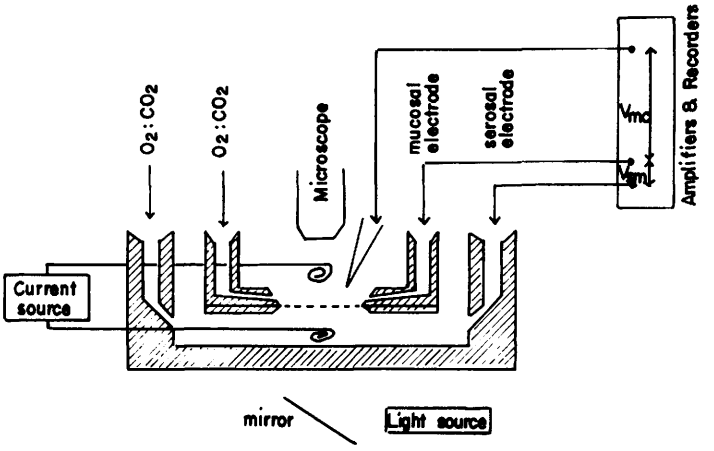

FIG. 1. Diagram of chamber in which the mouse jejunum was mounted. The dotted line represents the mesh used to support the intestine. The outside water jacket is not shown here.

resistance of the microelectrodes used varied from 5 to 30 Mohm and the tip potential was not more than 5 mV.

During the experiment a microelectrode was held on a KCl-agar bridge which made contact with a calomel electrode through a saturated KCl bridge. The microelectrode movement was controlled by a Leitz micromanipulator. The potential measurement was made in the following manner: After a stable transmural potential (V_{sm}) was attained, the microelectrode was manipulated into a surface mucosal epithelial cell, and a sudden drop of potential difference between the microelectrode and mucosal electrode was observed and recorded as V_{mc}. The microelectrode was then moved to a different site and measurements were repeated.

Results. The criteria for an acceptable intracellular potential measurement were: (i) a sudden negative drop in the potential of the microelectrode with respect to the reference electrode which was placed in the mucosal bathing fluid; (ii) a stable PD for at least 10 sec; (iii) no significant change in tip resistance while the microelectrode was inside the epithelial cell; (iv) a return to baseline measurement after withdrawal of the microelectrode; (v) no change in tip potential and resistance after the withdrawal. Due to the movement of intestinal villi and possible muscular contraction, only 25% of the cellular impalements were considered acceptable according to these criteria.

Intracellular potentials in glucose and glucose-free media. In all the experiments performed here, unless otherwise indicated, the intestine was bathed in 5.5 m*M* glucose containing Krebs-Ringer solution and the chamber was kept at a constant room temperature of 23°. The transmural potential, V_{sm}, which was recorded very steadily throughout the experiment, ranged from 1.6 to 4.2 mV, with the serosal side being electropositive to the mucosal side. The recorded intracellular potential, V_{mc}, ranged from 11 to 37 mV, with the intracellular side being electronegative to the mucosal side. The potential measurements remained stable for more than 2-5 min. In a total of 45 control studies, the average V_{sm} was 2.68 ± 0.09 mV (mean ± SEM), the average V_{mc} was 22.5 ± 0.87 mV, and the calculated V_{sc} was 25.2 ± 0.84 mV.

In 19 experiments where the bathing media was replaced with glucose-free Krebs-Ringer solution, the V_{sm} dropped from a control value of 2.59 ± 0.1 to 1.28 ± 0.07 mV, while the intracellular potential, V_{mc}, hyperpolarized from a control value of 21.8 ± 0.59 to 29.5 ± 0.53 mV. The change of the potentials was then calculated. The ΔV_{sm} was −1.31 mV, the ΔV_{mc} was +7.69 mV, and the ΔV_{sc} was 6.38. Such changes are statistically significant at the $P < 0.001$ level.

Effects of ethacrynic acid (EA), furosemide, and amiloride. EA was added to the glucose-containing mucosal bathing

solution at a concentration of 0.5 m*M*. It caused a drop in V_{sm} from a predrug control value of 2.73 ± 0.29 mV to a postdrug value of 1.80 ± 0.22 mV. The drop of V_{sm} was accompanied with an hyperpolarization of the intracellular potentials V_{mc} and V_{sc}. The V_{mc} increased to 34.8 ± 0.68 mV from a predrug value of 22.2 ± 1.88 mV and the V_{sc} increased to 36.6 ± 0.64 mV from a predrug value of 24.9 ± 1.91 mV. In another set of six experiments, after control V_{sm} and V_{mc} were recorded in glucose-containing Ringer solution, the bathing fluid in both sides was then replaced with glucose-free Ringer solution and new control values of V_{sm} and V_{mc} were recorded. Usually 30 min were allowed for reaching a steady sate, then EA was added to the mucosal solution. A decrease of V_{sm} was observed, but there was no further change of V_{mc} and V_{sc} as seen in the tissue which was bathed with glucose-containing solution. Results are summarized in Table I.

When either furosemide or amiloride was added to the mucosal bathing fluid, an effect similar to that of EA on V_{mc} and V_{sc} in glucose-containing media was produced. In glucose-free media, however, amiloride at a final concentration of 0.5 m*M* caused a further decrease of V_{sm} and a hyperpolarization of V_{mc}, resulting in a net change of 10 mV in V_{mc}. Although the addition of furosemide in glucose-free solution produced a less significant drop in V_{sm}, a significant hyperpolarization of V_{mc} was obtained and resulted in a net change of 11 mV in V_{mc}.

Effects of phlorizin and EA on intracellular potential. In a set of four experiments using glucose-containing media, control recordings of V_{sm} and V_{mc} were obtained. Then phlorizin at a final concentration of 0.5 m*M* was added in the mucosal side; a decrease in V_{sm} and an increase in V_{mc} were observed. The magnitude of the change in V_{mc} (ΔV_{mc}) was almost equivalent to that control value observed in glucose-free media, as shown in Table I. EA at a concentration of 0.5 m*M* was added to the phlorizin and glucose-containing bathing fluid. No further change in V_{mc} was observed, but there was a slight further decrease in V_{sm}.

Temperature Dependence. In a set of five experiments the intestine was bathed in glucose-containing media and the temperature dependence of V_{sm} and V_{mc} was studied. After stable recording at room temperature (23° or 296°K) was obtained, the temperature of the bathing chamber was lowered by placing ice in the water jacket and recorded through a temperature probe placed near the tissue. Over a period of 2 hr, the temperature went slowly from 296 to 280°K and then back to 296°K or room temperature. The relationships between the recorded V_{sm} and V_{mc} and temperature are summarized in Fig. 2. The data were fed to a computer for the fitting to six different linear and nonlinear

TABLE I. SUMMARY OF POTENTIAL MEASUREMENTS ACROSS THE MOUSE INTESTINE.

D-Glucose	Drug	V_{sm} (mV)	*P* value[a]	V_{mc}	*P* value	V_{sc}
5.5 m*M*	None (control)	2.68 ± 0.09 (45)[b]		22.5 ± 0.87		25.2 ± 0.85
5.5 m*M*	EA, 0.5 m*M*	1.80 ± 0.22 (8)	<0.01	34.8 ± 0.68	<0.001	36.6 ± 0.64
5.5 m*M*	Furosemide, 0.5 m*M*	2.30 ± 0.18 (6)	>0.1	31.9 ± 2.6	<0.05	34.2 ± 2.49
5.5 m*M*	Amiloride, 0.5 mM	1.90 ± 0.26 (8)	<0.01	32.5 ± 4.07	<0.05	34.5 ± 4.11
5.5 m*M*	Phlorizin, 0.5 m*M*	1.99 ± 0.18 (4)	<0.02	31.2 ± 0.92	<0.01	33.2 ± 1.01
5.5 m*M*	Phlorizin, 0.5 m*M* + EA, 0.5 m*M*	1.45 ± 0.2	<0.05	31.5 ± 0.86	>0.8[c]	32.90 ± 0.94
0	None (control)	1.28 ± 0.10 (19)		29.5 ± 0.53		30.8 ± 0.50
0	EA, 0.5 m*M*	0.92 ± 0.03 (6)	<0.01	28.5 ± 1.6	>0.8	29.4 ± 1.58
0	Furosemide, 0.5 m*M*	0.98 ± 0.15 (6)	>0.2	40.7 ± 0.64	<0.001	41.6 ± 0.56
0	Amiloride, 0.5 m*M*	0.89 ± 0.09 (7)	<0.01	39.8 ± 1.20	<0.001	40.7 ± 1.25

[a] *P* value was calculated by comparing the control data and the data obtained after addition of the drug.
[b] Mean ± SEM. Number of animal experiments is given in parentheses.
[c] This *P* value was calculated by comparing the phlorizin value and that after EA addition.

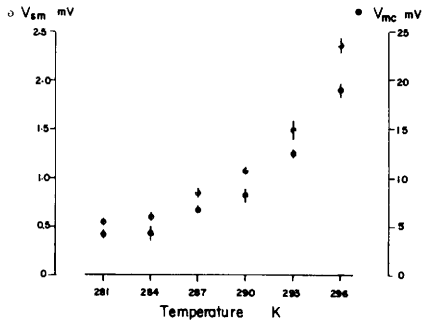

FIG. 2. Effect of temperature on the transmural and intracellular potential. Each point is the mean of six to eight measurements; the vertical bars are standard errors of the mean.

curves. It was found that an exponential correlation between the temperature and the potentials was indicated.

In order to calculate the activation energies and the Q_{10} values for the ionic transport across the intestine, E_A^{sm}, and for ions crossing the mucosal membrane barrier, E_A^{mc}, the Arrhenius plots of ln 10 V_{sm} vs $1/T$ and ln V_{mc} vs $1/T$ were used, as presented in Fig. 3. The activation energies were then calculated from the slopes of the straight lines that were obtained by the method of linear regression analysis. E_A^{sm} was found to be 19.32 kcal/mole and E_A^{mc} was 14.02 kcal/mole with Q_{10} values of 2.89 and 2.16, respectively. Using the relationship $V_{sc} = V_{mc} + V_{sm}$, the linear regression analysis of ln V_{sc} vs $1/T$ gave a calculation for E_A^{sc}, the activation energy across the serosal membrane barrier, of 14.49 kcal/mole. Table II summarizes these data and compares them with those of Quay and Armstrong (9) and Schultz and Zalusky (10) obtained from other animals.

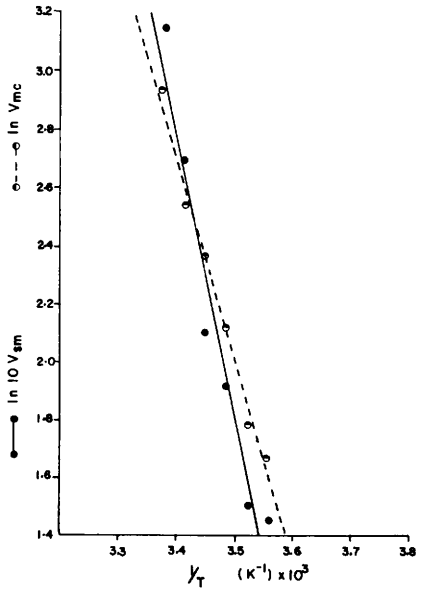

FIG. 3. Arrhenius plots of the effect of temperature on V_{sm} and V_{mc}.

Discussion. Table III summarizes the potential measurements of intestinal mucosa in various animal species obtained by others and by us. In the papers by Rose and Schultz (1), White and Armstrong (5), and Armstrong *et al.* (11), an equivalent electrical circuit for the small intestine of rabbit and frog has been presented. With that electrical analog, they explained qualitatively the effect of amino acids, sugars, and external osmolality changes on the transmural and intracellular potential differences. Their model consists of an emf with an internal resistance for each of the mucosal and serosal membranes and a parallel shunt pathway with another emf

TABLE II. TEMPERATURE COEFFICIENT OF IONIC TRANSPORT ACROSS THE INTESTINAL MUCOSA.

		E_A (kcal/mole)			Q_{10}			
Animal	Condition	V_{sm}	V_{mc}	V_{sc}	V_{sm}	V_{mc}	V_{sc}	Reference
Mouse	NaCl Ringer + 5.5 m*M* glucose	19.32[a]	14.02	14.49	2.89	2.16	2.22	
Rabbit	Glucose free	7.3			1.60			(10)
	11 m*M* Glucose	13.6			2.00			(10)
Frog	NaCl Ringer + 11 m*M* glucose	12.4[b]			2.00[c]			(9)
	Na_2SO_4 Ringer + 11 m*M* glucose	17.5[b]			2.7			(9)

[a] Data were calculated from ln 10 PD or ln PD vs $1/T$, as shown in Fig. 3. Temperature ranged from 281 to 296°K.

[b] Data were calculated from ln I_{sc} vs $1/T$. Temperature ranged from 285.2 to 307.4°K.

[c] Was not listed in Quay and Armstrong's (9) paper, but was calculated by the authors.

TABLE III. POTENTIAL MEASUREMENT OF INTESTINAL MUCOSA IN VARIOUS SPECIES.

Animal	Transmural V_{sm} (mV)	Mucosal or Intracellular PD V_{mc} (mV)	References
Rabbit ileum	4.5	36	(1)
Rat duodenum	2.1	53	(3)
Rat jejunum	10.6	9.4	(2)
Tortoise	12.3	6.6	(4)
Frog	6	35	(5)
Mouse	2.68	22.5	

to account for the transepithelial shunt resistance and the possibility of transepithelial diffusion potential that may develop from ionic gradients across the cell.

In previous work, our laboratory reported that a net absorption of Na^+ and secretion of Cl^- was observed in mouse small intestine which was bicarbonate dependent and augmented by glucose (6). Later, we showed that EA, furosemide, and amiloride inhibited Na^+ and Cl^- fluxes and also the residual flux (J^R_{net}) (7). The mechanism of the inhibition by EA is different from that of furosemide or amiloride. Data obtained from our present studies substantiates the conclusion that EA affects mainly the Na^+-glucose cotransport system in the mucosal membrane because it has little or no effect on V_{mc} in the absence of glucose or in the presence of phlorizin. The other two diuretics, furosemide and amiloride, do exert a hyperpolarizing effect on V_{mc}, regardless of the presence or absence of glucose, suggesting they affect mainly the membrane conductance to Na and/or Cl ion. However, with various pathways being affected differently, it is difficult to use the model of Rose and Schultz (1) or that of White and Armstrong (5) to qualitatively explain such findings on diuretic effects in electrolyte fluxes and intracellular potential measurements, since only the net effect could be defined from their model and not the individual contributions. We, therefore, propose a model, as diagrammed in Fig. 4, in which the individual ionic pathways, pumps, and gradients are more explicitly defined, especially on the mucosal side which is our primary concern. Our model shows Na^+ entering the mucosal membrane through two pathways (12) which are represented in the upper two limbs. The first limb is a diffusion limb and

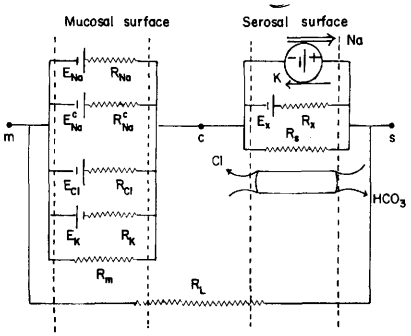

FIG. 4. Diagrammatic presentation of a proposed electrical model in intestinal mucosa. (m) mucosal side, electronegative to serosal side (s); (c) cellular side, negative to mucosal and serosal sides; (R_m), (R_s), and (R_L) are resistance shunts across mucosal and serosal membranes and tight junction, respectively.

the second is the cotransport limb of Na^+-glucose or Na^+-amino acids. Since there is a process of active Cl^- secretion in mouse intestine (6), the third limb in our model represents the Cl^- secretion and the fourth is a potassium diffusion potential limb. R_m is the shunt across the mucosal membrane. As for the serosal membrane, a Na-K ATPase pump is depicted (13, 14), however, since an exchange pump could be either neutral or electrogenic depending on the coupling ratio, we have added to the usual notation of the pump the emf symbol ($-\ \|+$) to indicate that the Na-K ATPase pump here is electrogenic (15, 16). The existence of Cl-HCO_3 neutral exchange (6) is displayed in the last limb. The other possible ionic pathways, including the K leak, are pooled together by the terms E_x and R_x, while R_s represents the serosal membrane shunt and R_L the transepithelial shunt. The presence of the K- and Na-passive pathway on the mucosal side is supported by the work of Okada *et al.* (3) who found that changes in K or Na concentration in the mucosal bathing solution would introduce a change in V_{mc}, while change in Cl^- concentration did not affect the V_{mc} measurement. From the circuit as presented here, one can solve for V_{mc} and V_{sc} as follows:

$$V_{mc} = \frac{(E_K G_K - E_{Na} G_{Na} - E^c_{Na} G^c_{Na} - E_{Cl} G_{Cl}) \cdot (G_L + G_s + G_x) - E_x G_x G_L}{(G_{Na} + G^c_{Na} + G_{Cl} + G_K + G_M) \cdot (G_L + G_s + G_x) - G_L^2} \quad [1]$$

and

$$V_{sc} = \frac{E_x G_x + V_{mc} G_L}{G_x + G_s + G_L}. \quad [2]$$

Results obtained from others (4, 5, 17) and from our present studies demonstrate that glucose depolarizes the intracellular potential and increases the transmural potential. Based on Eq. [1] it is easy to explain such phenomena as when G^c_{Na} increases, the V_{mc} decreases, and vice versa. By adding phlorizin to glucose-containing bathing solution, the glucose transport is inhibited and the G^c_{Na} is also inhibited resulting in an increase of V_{mc} or a hyperpolarization.

EA was found to inhibit Na^+ absorption, Cl^- secretion, and glucose and amino acid transport across the mouse intestine (7). Such an effect could be interpreted as a decrease in $E_{Na}G_{Na}$, $E^c_{Na}G^c_{Na}$, and $E_{Cl}G_{Cl}$, resulting in an increase of V_{mc} or hyperpolarization. However, when EA was added either to glucose-free bathing media or glucose Ringer solution with phlorizin added, where $E^c_{Na}G^c_{Na}$ is practically zero, no further hyperpolarization of V_{mc} was observed. The conclusion is, therefore, that EA mainly affects the glucose-coupled sodium transport system and the Cl^- pathway at the mucosal side. However, the addition of EA to the glucose-free medium or to the glucose-containing medium pretreated with phlorizin did decrease the transmural potential, V_{sm}, with no significant changes in V_{mc} and V_{sc}. Therefore, it is difficult to rule out the possibility that EA may also have an effect, although at a much lower extent, on the sodium diffusive pathway, G_{Na}, or perhaps on the transepithelial shunt, G_L, because a small change in G_L will reflect a larger change in V_{sm}, rather than V_{mc} or V_{sc}. Chez *et al.* (8) studied the effect of EA on rabbit ileum and found that EA increased Na^+ influx across the brush border of the mucosal membrane of the ileum and the effect on Na absorption by EA was explained by the inhibitory effect on the active Na^+ transport pump at the serosal side. However, the authors did not furnish an explanation of why EA produced less inhibitory effect when placed in the serosal fluid than in the mucosal fluid. In two preliminary experiments in which ouabain was added to the medium at a concentration of 10^{-4} *M*, no effect on either V_{sm} or V_{mc} was observed when it was added to the mucosal side, while adding it to the serosal side caused a decrease in V_{sm} from 3 to 1 mV and a depolarization of V_{mc} from 28 to 14 mV. If EA does increase the Na^+ flux on the brush border, one should be able to measure a depolarization in the mucosal membrane potential and not an hyperpolarization as we obtained here. Whether such a difference between our results and those of Chez *et al.* (8) could be attributed to species differences we can not answer here. However, Pichon and Treherne (18) studied the intraepithelial potential on cockroach central nervous connectives and found that EA induced a hyperpolarization in the intact connectives and thereby substantiates our data.

The other two diuretics, furosemide and amiloride, inhibited Na^+ and Cl^- transport and had no effect on either glucose or amino acid transport (7). Fig. 5 presents a comparison of the effects of these three diuretics on potential measurements. As seen here, both furosemide and amiloride increased the intracellular potential, regardless of whether the bathing media were glucose-containing or glucose-free. The results suggest that the effects of furosemide and amiloride are mainly on the membrane ionic conductance and not on $E^c_{Na}G^c_{Na}$.

As for the effect of temperature on the transmural and intracellular potentials, the data presented in this study clearly indicate the dependence of these parameters on temperature, as one would expect for energy-mediated processes. From the variation of the short circuit current with temperature, Quay and Armstrong (9) calculated the activation energy E_A for ion transport in the isolated bullfrog small intestine in sulfate and chloride media and found that the E_A varied between 12.4 and 17.5 kcal/mole, with Q_{10}'s of 2 and 2.7, respectively. Similarly, Schultz and Zalusky (10) calculated the activation energy of rabbit ileum in the presence or absence of glucose as 13.6 and 7.3 kcal/mole, with Q_{10}'s of 2.0 and 1.6, respectively. Data obtained in our present studies, as shown in Table II, give the calculated activation energy, $E_A{}^{sm} = 19.3$ kcal/mole, with $Q^{sm}_{10} = 2.89$, which seems higher than that obtained by the other investigators. Such a difference could be attributed

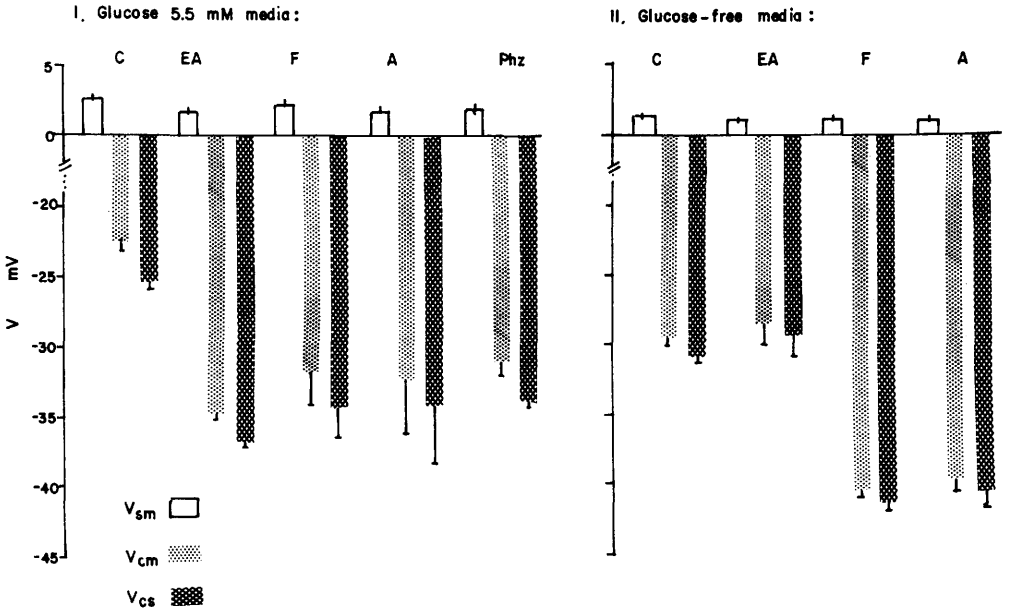

FIG. 5. Comparison of the effects on transmural and intracellular potentials produced by three diuretics and phlorizin. (EA) ethacrynic acid; (F) furosemide; (A) amiloride; (Phz) phlorizin. Each column represents the mean ± 1 SEM.

to the small number of experiments conducted in these three studies. However, Quay and Armstrong (9) and Schultz and Zalusky (10) used the plot of short-circuit current vs $1/T$ and did not provide the variation of the intracellular potential with temperature as we measured here. Furthermore, it should be pointed out here the difference between the activation energy for the potential across the intestine, E_A^{sm} = 19.3 kcal/mole, and for that across the mucosal membrane, E_A^{mc} = 14.02 kcal/mole and across the serosal membrane, E_A^{sc} = 14.49 kcal/mole. The difference in the activation energy of E_A^{sm} on one hand and those of E_A^{mc} and E_A^{sc} on the other hand indicates the presence of an important coupling between processes on both the mucosal and serosal membranes. While this coupling could be provided by the transepithelial shunt, G_L, it may also be present within the cell unit via intracellular carriers.

Summary. To substantiate our previous hypothesis in explanation of the inhibitory effect of EA, furosemide, and amiloride on mouse intestine, the intracellular potential, V_{mc}, and transmural potential, V_{sm}, of the intestinal mucosal epithelium were measured simultaneously with a 0.5-μm diameter microelectrode. It was found when glucose was present in the Ringer bathing solution, an average V_{mc} of 22.5 ± 0.87 mV was recorded and V_{sm} was 2.68 ± 0.09 mV. EA, furosemide, and amiloride at 0.5 m*M* produced an hyperpolarization of V_{mc} and a reduction of V_{sm}. The V_{mc} increased to 34.8 ± 0.68, 31.9 ± 2.6, and 31.2 ± 0.92 mV, respectively. When the bathing fluid contained no glucose, the average V_{mc} was 29.5 ± 0.53 mV and the average V_{sm} was 1.28 ± 0.10 mV. Addition of EA in the glucose-free bathing media caused no significant change in V_{mc}, but a decrease in V_{sm}. However, addition of furosemide or amiloride did cause further hyperpolarizations of V_{mc}, to 40.7 ± 0.64 and 39.8 ± 1.2 mV, respectively. These results support our conclusion that EA mainly acts on the Na-glucose cotransport system and the other two diuretics act on the membrane conductance. The Arrhenius plots of the variations of V_{sm} and V_{mc} with temperature provide the calculation of the activation energies for the transmural and transmucosal potentials as 19.3 and 14.02 kcal/mole, respectively.

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