TABLE 2

Appearance of precursor label in amino acids synthesized by isolated skate tissues

Precursor	Amino acid	Tissue	dpm/g Incubation 1*	dpm/g Incubation 2**
^{35}S-sulfur	Taurine	Liver	ND	Trace
		Brain	ND	Trace
		RBC	ND	Trace
		Heart	ND	Trace
^{14}C-cystine	Taurine	Liver	ND	-
^{14}C-choline chloride	Sarcosine	Liver	Trace	2010
		Kidney	Trace	6103
		Muscle	-	Trace
	Glycine	Liver	-	1317

Values are the means of duplicate chromatographic runs from one fish.
Trace activity indicates experimental cpm < 2 x background cpm.
ND = not detectable.

*Incubation medium was elasmobranch saline that contained 0.1 mM labeled precursor (20 µCi/ml).

**Incubation medium was elasmobranch saline that contained 0.1 mM labeled precursor (20 µCi/ml) and 10.0 mM amino acid end product.

slices to synthesize BALA. Thus, unlike the degradation and membrane transport of BALA, synthesis of this amino acid does not appear to be regulated during acclimation to a dilute environment.

Five metabolic pathways have been identified for taurine synthesis in biological systems (Jacobson and Smith, Physiol. Rev. 48(2):424-511, 1968). Our study tested four of the five pathways and significant taurine synthesis could not be demonstrated in any of the tissues. Considering the high levels of taurine in the skate and the lack of evidence supporting *de novo* synthesis of this amino acid, a high dietary source of taurine or its synthesis in the gut by bacteria may be important.

The present study also provides information concerning sarcosine degradation. We have previously been unable to test sarcosine oxidation due to low sensitivity of nonradioactive assays and the unavailability of an appropriately labeled preparation of the amino acid. The results of the present work indicate that the skate liver has the capacity to synthesize glycine from choline chloride thus providing evidence for sarcosine metabolism: choline ⟶ sarcosine ⟶ glycine. This work was supported by NSF grant PLM 75-14322.

ELECTROPHYSIOLOGICAL STUDY OF THE APICAL BARRIER OF THE INTESTINAL EPITHELIUM OF WINTER FLOUNDER (*Pseudopleuronectes americanus*)

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The intestinal epithelium of the winter flounder (*Pseudonectes americanus*) absorbs NaCl by a process that is thought to involve a neutral uptake of NaCl at the apical (mucosal) barrier of the cells (Field et al., J. Memb. Biol. 41:245, 1978, and Frizzell et al., J. Memb. Biol., in press). To characterize the apical barrier electrophysiologically the voltage at the apical barrier (V_a) was determined with intracellular microelectrodes and the changes of voltage observed in response to alteration of the ionic composition of the mucosal solution. Of particular interest was the finding that changes of mucosal potassium concentration caused large depolarizations of the V_a while changes of Na and Cl concentrations had either little or paradoxical effects on the V_a.

Methods

Pieces of intestinal epithelia stripped of their muscularis mucosa were mounted horizontally in special chambers so that the mucosal surface of the tissue could be penetrated with microelectrodes advanced perpendicularly to the tissue from above. Both mucosal and serosal solutions were allowed to flow continuously through the chambers so that changes of fluid composition could be made without disturbing the microelectrodes while monitoring both the intracellular voltage (V_a) and the transepithelial voltage (V_T). The composition of the fluids are given in Table 1 and will be referred to as either control, low NaCl, low Na, low Cl or high K. All substitutions were made on an equimolar basis with tetramethylammonium (TMA) replacing Na and with methyl sulfonate or isethionate replacing chloride. The temperature of all solutions was maintained at 15 ± 0.5°C.

Table 1

Solutions

	Control	low NaCl	low Na	low Cl	high K
Na^+	165	0	0	165	0
K^+	5	5	5	5	170
choline$^+$	-	15	15	-	-
TMA$^+$	-	150	150	-	-
Mg^{++}	1	1	1	1	1
Ca^{++}	1	1	1	1	1
Cl^-	152	0 or 2	152	0 or 2	152
HCO_3^-	20	20	20	20	20
methyl sulfonate$^-$	-	150	-	-	-
$SO_4^=$	1	1	1	1	1
isethionate$^-$	-	-	-	150	-
glucose (serosa)	10	10	10	10	10
mannitol (mucosa)	10	10	10	10	10

All solutions gassed with 99% O_2, 1% CO_2. All values are in mmole/L.

Microelectrodes were pulled from Omega dot glass capillaries (30-30-1, Frederick Haer & Co., Brunswick, Maine) filled immediately before use with 3 M KCl. In some cases the electrodes were filled with either 150 mM KCl or 1 M Na citrate and no differences were observed in the values of V_a. The microelectrodes were advanced in steps of 1 µM with a Narishege stepping motor drive (Model ME-71). The tissues were easily penetrated with microelectrodes. Indeed, after initial touch of the tissues, cells were penetrated cleanly within a few microns of advancing the electrodes. Thereafter the voltages usually remained within ± 1 to 2 mV for considerable periods of time often exceeding 5 to 10 minutes or more. Repeated punctures of the tissue gave similar values of V_a with standard errors of the mean near ± 1 to 2 mV (see Table 2).

The animals were caught locally and were stored in running seawater before use for several days without feeding. All voltages were measured with reference to a grounded mucosal solution. Accordingly, in this tissue the spontaneous V_a was normally cell interior negative to the mucosal solution. To indicate directional changes of voltage the terms "hyperpolarize" and "depolarize" will be used to indicate an increase and decrease of the magnitude of the spontaneous voltage observed under open-circuit conditions. No attempt was made in these studies to short-circuit the tissues. Indeed, with values of V_T near zero, the tissues could at least to a first approximation be considered to be self-short-circuited.

Table 2

Summary Values of Apical Membrane (V_a) and Transepithelial Voltage (V_T)

Control V_o	Flounder Intestine V_a, mV	V_T, mV
7/ 6/78	-62.7 ± 1.9 (7)	-2.9
7/11/78	-55.9 ± 1.0 (11)	-1.1
7/12/78	-52.6 (2)	-1.0
7/13/78	-69.6 ± 1.6 (17)	-3.8
7/18/78	-57.7 ± 1.3 (9)	-1.6
7/19/78	-66/9 ± 2.3 (5)	-3.8
7/20/78	-64.3 ± 2.8 (3)	-1.8
7/21/78	-58.5 ± 2.5 (5)	-4.2
7/22/78	-66.1 ± 1.4 (14)	-3.6
Mean ± SE	-61.6 ± 1.9 (9)	-2.9 ± 0.4 (9)

Results

The spontaneous values of V_a and V_T in 9 tissues are summarized in Table 2 for tissues incubated with the control solution in both mucosal and serosal chambers. The population means for V_a and V_T were -61.6 and -2.9 mV, respectively.

Ion Substitutions on V_a: When the NaCl concentration of the mucosal solution was decreased from 165 to 0 mM, the V_a was increased (hyperpolarized) by approximately 9 mV. In one tissue, the TMA methyl sulfonate was substituted directly and isosmotically with sucrose resulting in no change of the V_a. Thus, the change of V_a with low NaCl substitution could be attributed to a decrease of the NaCl concentration of the mucosal solution $[NaCl]_m$. To investigate this further, single ion substitutions were done: TMA for Na and isethionate for Cl. As shown in Table 3, reduction of $[Na]_m$ had no effect on the V_a while reduction of $[Cl]_m$ had a similar effect on V_a as observed with reduction of $[NaCl]_m$.

Whereas these effects on V_a could be attributed to a change of $[Cl]_m$, it should be noted that the changes of V_a were relatively small, near 11%. The directional changes were paradoxical in the sense that a decrease of $[Cl]_m$ would be expected to depolarize rather than hyperpolarize the V_a if Cl transport occurred via a conductive mechanism.

Table 3

Effect of Mucosal Ion Substitutions on V_T and V_a

	V_a, mV			V_T, mV		
	Control	Experimental	ΔV_a	Control	Experimental	ΔV_T
low $[NaCl]_m$ (N=4)	-51.7 ± 0.9	-70.6 ± 1.9	8.9 ± 2.6	-3.5 ± 0.4	-17.8 ± 1.7	14.1 ± 2.1
low $[Na]_m$ (N=3)	-63.7 ± 2.2	-64.6 ± 2.0	0.8 ± 0.3	-1.7 ± 0.4	- 5.4 ± .4	3.7 ± 0.8
low $[Cl]_m$ (N=6)	-58.1 ± 3.5	-64.4 ± 3.5	6.4 ± 2.0	-1.0 ± 0.2	- 1.4 ± 0.8	0.4 ± 0.7
high $[K]_m$ (N=11)	-58.4 ± 2.0	- 5.4 ± 1.9	-53.8 ± 2.2	-1.6 ± 0.4	+ 8.5 ± 1.5	-10.0 ± 1.7

Values are Mean ± SE. mV.

Elevation of mucosal [K] caused large depolarizations of the V_a. Indeed, as shown in Table 3, elevation of $[K]_m$ from 5 to 170 mM caused the V_a to decrease by 53.8 ± 2.2 mV. Such a depolarization of the V_a is compatible with the notion that the apical barrier is highly permeable to K. Since it was possible that elevation of [K] caused depolarization of V_a by an effect at the serosal barrier of the cells (K appearing in the lateral intercellular spaces via leaks through the tight junction), the serosal [K] was elevated from 5 to 170 mM as was done for mucosal K substitutions. Although V_a was observed to decrease in 2 tissues from -67.1 to -33.0 and -57.4 to -35.0 mV, the changes of V_a were considerably less than observed for mucosal K substitution alone. Thus, it is likely that elevation of mucosal [K] on the V_a can be attributed to its effect at the apical barrier of the cells.

Ion Substitutions on V_T: Owing to the very low electrical resistance of the tissue (~ 40 Ω cm^2, Frizzell et al., J. Membrane Biol., in press) and its low spontaneous value of V_T in the presence of large intracellular voltages, it is likely that this tissue possesses low resistance shunt pathways that to a large extent influence the magnitude of the V_T. Thus, to a first approximation, changes of V_T in response to a change of ionic composition might be used to determine qualitatively the relative permeability to ions of the shunt pathway. When $[Cl]_m$ was reduced from 152 to 0 mM, no change of V_T was observed. However, reduction of $[Na]_m$ caused hyperpolarization of V_T, and an increase of $[K]_m$ caused depolarization of the V_T as would be expected if the shunt pathways were primarily permeable to these cations. No attempt was made in these studies to determine the concentration-dependence of the V_a and V_T. In addition, the changes of V_T could in part also reflect changes of conductance of the shunt pathway with ion substitutions and in part the IR drop in the shunt pathway subsequent to changes of ionic composition would also contribute to changes of V_T due to changes of circulating currents. Thus, no firm conclusion can be made from these data except to indicate that the V_T is in part dependent on the concentrations of Na and K but not Cl in the mucosal solution.

Discussion

Whereas the intestinal epithelium of the flounder absorbs NaCl, its mechanism is thought to include a neutral uptake of NaCl from the mucosal solution. The data of the present studies would seem to be in accord with this view since changes of $[Na]_m$ and $[Cl]_m$ caused little or no changes of the V_a that would be expected if Na and/or Cl transport occurred via electrically conductive mechanisms. Of particular interest, however, was the finding that the V_a was quite sensitive to changes of $[K]_m$. Indeed, if the apical barrier is highly permeable to K, then for K to be in equilibrium at the apical barrier the intracellular concentration of K would be expected to be near 64 mEq/L assuming a $[K]_m$ of 5 mEq/L and a V_a of -62 mV. Values of intracellular K activity are presently unknown. However, if K is not at equilibrium at the inner barrier, then it remains possible that the apical barrier may serve as a site for either K absorption and/or secretion.

Although not reported in detail here, studies were also attempted of the operculum of the killifish (*Fundulus heteroclitus*). Using identical procedures we found it impossible to obtain successful

impalements of the cells of this epithelium with the microelectrodes available. In contrast, in one study of the rectal gland of the spiny dogfish (*Squalus acanthias*) cellular impalements of slices of tissues gave stable negative intracellular potentials between 90 and 103 mV. (Supported by USPHS AM 16663, National Institute of Arthritis, Metabolism and Digestive Diseases.)

MECHANICS OF THE SINGLE CELL LAYERED HEART OF "SEA POTATO"

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The heart of the "sea potato" is a tubular structure which consists of a single layer of cells. Previous experiments from our lab have shown that laser diffraction methods (Cleemann, Dillon and Morad, MDIBL Bull., 16:8-13, 1976), can be used to measure the sarcomere length from a sheet of myocardial tissue. The sheet is fastened along the perimeter of a rectangular aperture and a servo-control device was used to bulge-up the tissue and keep it length clamped during the time course of contraction. In the experiments described below the measurements of sarcomere dynamics were continued and the contraction response to rapid stretch and release were recorded. These measurements obtained from a small segment of the myocardial wall were then compared to measurements obtained using the intact tubular heart.

Measurement of Sarcomere Dynamics: In 1976 we succeeded in measuring the passive and active sarcomere length-tension relationship in this tissue. In order to follow and servo-control the sarcomere length during the contraction the instrumentation was altered to increase the angular resolution of the light detector. Figure 1 panel A shows a diffraction pattern obtained after such improvements. The width of the central diffraction peak corresponds to a sarcomere length change less than 0.1 μm, suggesting an improvement over previous measurements. As an experiment progressed, it was consistently noticed that the diffraction peaks become broader and less defined as the twitch tension decreased. These observations suggest that the isolated sheeth preparation deteriorates because of cell injury and that the absence of signal was not limited by the resolution of the optical instrumentation.

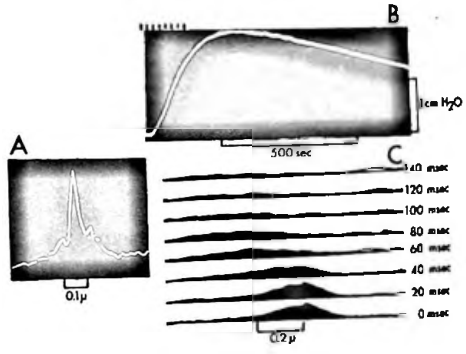

Figure 1. Measurement of sarcomere length using diffraction patterns. Panels A and B show diffraction patterns, i.e., the intensity of light as function of the scattered angle. The left side of the panels corresponds to a large diffraction angle and a short sarcomere length and the right side corresponds to a small diffraction angle and long sarcomeres. The diffraction patterns in panel C are measured at the points indicated during the twitch pressure shown in panel B. The diffraction patterns in panel C have been traced from oscilloscope recordings and are shown with subtraction of a common baseline scatter.