

measurements made under the same circumstances by other means, such as pH-sensitive microelectrodes or the equilibrium distribution of a weak base, such as nicotine. In addition, the possibility that flounder intestine secretes HCO_3^- when pH_o is 8.0 needs to be evaluated. In prior studies, the absence of a residual ion flux ($I_{sc} - J_{\text{net}}^{\text{Na}} + J_{\text{net}}^{\text{Cl}}$) suggested an absence of HCO_3^- secretion, but residual flux measurements are by their nature extremely inaccurate. This work was supported by NIH grant AM-21345 and NIH postdoctoral fellowship AM-05973 to P. L. Smith.

PRELIMINARY OBSERVATIONS ON MORPHOLOGY AND ELECTRICAL PROPERTIES OF GALLBLADDER EPITHELIUM FROM THE WINTER FLOUNDER, *PSEUDOPLEURONECTUS AMERICANUS*

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We report here our initial experiments with isolated gallbladder epithelium from the flounder. Methods and Ringer solution (20mM HCO_3^- -Ringer) were the same as those we have previously employed for flounder intestine (Field et al, J. Membrane Biol. 41:265, 1978). The Ringer solution was gassed with either 1% CO_2 in O_2 (pH 8.0) or 5% CO_2 in O_2 (pH 7.22). The gallbladder was stripped of muscle with fine, curved forceps. For morphology, gallbladder mucosa was fixed in gluteraldehyde immediately after removal from the Ussing chamber; it was post-fixed in osmium tetroxide and embedded in epoxy resin. Sections were cut $1\mu\text{m}$ thick and stained with toluidine blue.

After mounting, the PD across the gallbladder increased (in absolute value) steadily for 20-40 min, stabilizing at -6 to -9mV, mucosal reference. The resistance was 50 to $70 - \Omega \cdot \text{cm}^2$. Serosal addition of theophylline (5mM) alone or together with 8-Br-cAMP (0.2mM) decreased the PD down to -1 to -2mV gradually over 20-30 min. Reduction of medium pH from 8.0 to 7.2 caused a similar decline in PD. Dilution potentials were the same under control conditions, after theophylline and 8-Br-cAMP, and after acidification: 10% dilution of salts in the mucosal medium with isotonic mannitol produced an abrupt -2.0mV change in PD; serosal dilution gradually reversed the prior PD change (15-20 min). Selectivity ratio (R) of monovalent cations to monovalent anions was calculated from the constant-field equation as follows:

$$R = (e^{(F/RT) \Delta PD} [-]_m - [-]_s) / ([+]_m - e^{(F/RT) \Delta PD} [+]_s)$$

where $[-]$ and $[+]$ are the concentrations of monovalent anions and cations respectively and the other symbols have their usual meanings. Cation selectivity of the mucosa was about 8:1.

As shown in Figure 1, the cells are long ($\sim 55\mu\text{m}$) and narrow ($\sim 4\mu\text{m}$) with centrally to basally located nuclei and infrequent goblet cells.

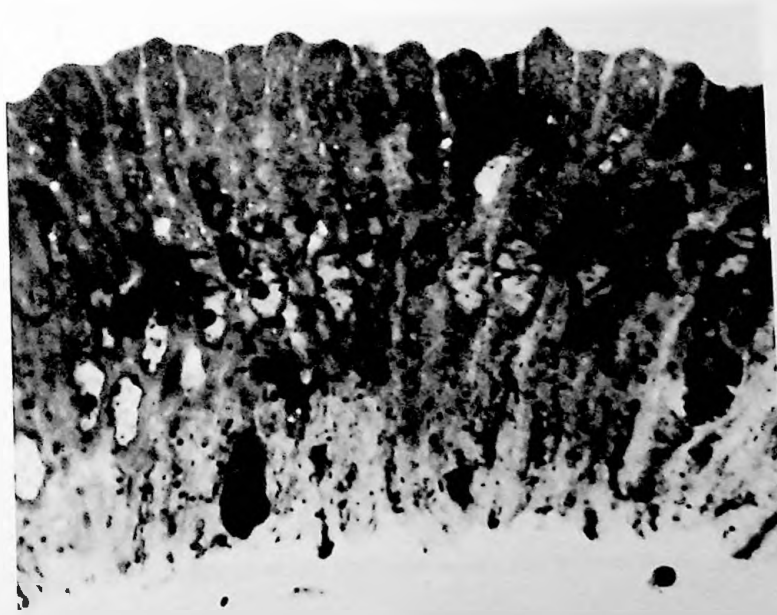


Figure 1. Light micrograph of flounder gallbladder magnified 1300X.

Flounder gallbladder, therefore, appears to have essentially the same ion transport properties as flounder intestine, namely, a serosanegative PD, which is inhibitable by cyclic AMP and also by a reduction in medium pH, and a high degree of cation selectivity in the paracellular pathway. Gallbladder resistance is about 50% higher than intestinal resistance. Morphologically the cells also appear similar in that they are both unusually long and narrow.

IDENTIFICATION OF AN INTRACELLULAR UPTAKE SYSTEM FOR TAURINE IN ATRIAL MYOCARDIUM OF THE SKATE, Raja erinacea

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The object of the current study is to establish values for the distribution of total tissue water, and the concentrations of taurine therein, between extracellular and intracellular regions in isolated "hemi-atria" of skate hearts. This is a continuation of last summer's work attempting to characterize a carrier mediated taurine transport system in skate myocardium. Expressing taurine concentrations on an intracellular rather than on a unit per total wet weight basis becomes particularly important when agents are used selectively to elucidate the cellular taurine transport system that simultaneously bring about a redistribution of intra- and extracellular water within the in vitro fragment during incubation (Forster, R.P. et al., Bull. Mt. Desert Is. Biol. Lab. 15: 1-4, 1977).

Taurine has been implicated in cell volume regulation in various tissues of a wide variety of invertebrates and vertebrates. In addition, its role in mammalian cardiac function derives from the observation that there is an increase in taurine uptake associated with various aspects of cardiac stress (Read, W.O. and J.D. Welty. J. Pharmacol. Exper. Therap. 139: 283-289, 1963). β -adrenergic stimulation has been shown to increase the transport capacity of taurine in rat hearts (Huxtable, R. and J. Hubb. Science 198: 409-411, 1977). Taurine transport was mediated by a β -amino acid uptake system in organ culture of fetal mouse hearts that was sodium dependent and capable of accumulating taurine against a concentration gradient (Grosso, D.S., Roeske, W.R. and Bressler, R.J. Clin. Invest. 61: 944-952, 1978).

The skate was used in this investigation because, as with all elasmobranchs, its heart has no sympathetic adrenergic supply and thus is an interesting model to test the previously suggested relationship in mammals between β -adrenergic stimulation and increased taurine influx (Burnstock, G. Pharmacol. Rev. 21: 247-324, 1969). Furthermore, the in vitro hemi-atrium preparation is an extremely viable preparation for use in transport studies generally, and Raja erinacea heart has the highest taurine values reported in any vertebrate species.

Methods

Procedure is generally the same as that we described in the 1978 Bull. Mt. Desert Is. Biol. Lab. 18: 1-4. The separated thin-walled atria were divided in half, blotted, weighed, and the separate samples then placed in Erlenmeyer flasks and preincubated in balanced isotonic medium for 15 min at 15°C. The hemi-atria were then transferred to new flasks containing elasmobranch medium and incubated for 1 hr under control conditions. In all cases, paired analyses were carried out with one hemi-atrium used as control while the other was subjected to the experimental variable. Details are included with the presentation of results. Control elasmobranch medium had the following concentration in millimoles per liter: 280 NaCl, 6 KCl, 5 CaCl₂, 3 MgCl₂, 0.5 Na₂SO₄, 1 NaH₂PO₄, 450 urea; these diluted approx. to 1 liter, and 8 NaHCO₃ then added dry while stirring. Five millimoles/liter of glucose were included on the day of use. Vessels were gassed with 99% O₂, 1% CO₂; pH 7.4.

Results and Discussion

The viability of the skate hemi-atrium preparation was further attested to by the uniformity of extracellular fluid space (ECS), measured as the percentage volume of distribution of [¹⁴C] inulin. Table 1 demonstrates the