TABLE II

Number of Dogfish Collected per Fishing Trip for each Week of the Summer Season

Week	1973	1974	1975	1976+	1977	1978	1979	1980	1981°	1982°°	1983	Average 1973-83++
1*	6	13	13	13	23	16	6	26	16	1	10	13 ± 7
2	18	31	9	17	32	7	14	10	20	10	26	18 ± 9
3	42	41	86	24	21	25	28	48	28	12	40	37 ± 20
4	69	48	31	29	48	25	39	31	50	11	36	39 ± 16
5	84	52	62	39	52	41	31	43	21	7	75	47 ± 24
6	50	55	53	30	33	26	10	41	43	21	32	36 ± 15
7	98	87	72	27	61	53	46	39	10	21	54	54 ± 27
8	65	62	54	38	37	75	27	52	19	15	66	47 ± 21
9	61	50	61	24	36	32	43	85	27	17	56	47 ± 20
10	53	29	35	6	41	25	32	60	50	18	66	41 ± 16
11**	-	8	-	-	6	40	12	31	44	11	33	23 ± 15

*Week number 1 includes all the collecting trips during the month of June.

**Week number 11 includes all the collecting trips during the month of September.

+The number shown for the year 1976 depicts only those fish delivered to individual investigator live cars. The total number collected was not available.

++Does not include the 1976 numbers.

°Includes two deliveries by Gordon King on week 2 and 5 and three deliveries on week 7; one delivery by Bud Hodgkins on week 10.

°°Includes one delivery by Gordon King on weeks 1 and 4, and two deliveries on week 5; and three deliveries by Bud Hodgkins on week 3.

THIAZIDE DIURETICS INHIBIT NaCl ABSORPTION BY THE FLOUNDER URINARY BLADDER

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The flounder urinary bladder absorbs NaCl by a neutral mechanism. The short circuit current (I_{sc}) present in some tissues is due entirely to K secretion and can be inhibited by administration of barium to the mucosa (Dawson and Andrew, MDIBL 20:89-92, 1980). Results presented previously indicate that the NaCl absorption process requires both Na and Cl for net transport. Net transport is dependent on an intact basolateral membrane Na-K ATPase and thus can be inhibited with ouabain. Previous results have indicated that the entry step does not require lumen potassium, is not sensitive to amiloride or DIDS applied to the luminal membrane, and is minimally sensitive to furosemide (Stokes and Lee, Bull., MDIBL 22:5-7, 1982). Thus, the cellular entry mechanism for NaCl is not via a Na:K:2Cl transport system nor via parallel Na:H Cl:OH exchangers.

Urinary bladders were dissected from flounder which had been maintained in tanks of flowing seawater for up to 10 days. The bladder was opened longitudinally and mounted in plastic rings in Ussing chambers as previously described by Dawson and Andrew (Bull. MDIBL 19:46-49, 1979). All measurements were made under short-circuited

conditions and transepithelial conductance (G_T) was measured by periodically passing sufficient current to clamp the voltage to 10 mV for 0.5 sec. The mucosal and serosal bathing solutions contained (in mM) NaCl, 140; KCl, 2.5; $CaCl_2$, 1.5; $MgCl_2$, 1.0; NaHEPES, 7.5; HEPES, 7.5; and glucose 5.0. Solutions were gassed continuously with room air so that pH equalled 7.5. As previously described, all tissues were exposed to 50 μM verapamil during mounting and during the flux studies. This concentration of verapamil reduces the contractions of the muscular layer and thus prevents spontaneous fluctuations in electrical activity but has no effect on ion transport. Unidirectional fluxes (J) of Na and/or Cl were conducted using the appropriate isotopes by standard techniques.

Table 1.--Effect of 1% CO_2 on Transport Characteristics of the Flounder Urinary Bladder

	Control	1% CO_2	Ouabain + Papaverine
I_{sc} ($\mu A/cm^2$)	2.0	2.0	0
	±1.6	±1.6	
G_T(mS/cm^2)	0.38	0.36	0.40
	±0.06	±0.05	±0.06
J^{ms}_{Na} ($\mu Eq/cm^2h$)	1.11	1.28*	0.65*
	±0.20	±0.18	±0.08

Values are mean ± SEM.

CO_2 was gassed into serosal chamber. Ouabain (0.1 mM) added to serosa, papaverine (50 μ M) added to mucosa (n=4).

Table I demonstrates the effect of raising pCO_2 to 8 mM of Hg, acidifying the serosal solution (to 6.8) as well as the cell. Mucosal solution was bubbled with room air. There were no changes in I_{sc} or G_T. J^{ms}_{Na} increased slightly. The ouabain and papaverine treatment period indicates the magnitude of the passive component of Na transfer for each tissue.

Preliminary studies indicated that thiazide-type diuretics might inhibit NaCl transport in this tissue. A thorough evaluation of the actions of hydrochlorothiazide indicated that: a) mucosal addition of hydrochlorothiazide (0.1 mM) completely inhibited net Na absorption, b) it was not effective when added to the serosal solution, c) the I_{sc} when present was reduced to near 0, and d) G_T invariably increased. Examination of the dose-response to hydrochlorothiazide on the electrical parameters indicated that half-maximal activity was approximately 2-5 x 10^{-5} M. The results of hydrochlorothiazide application to the mucosal solution are depicted in Table 2.

These experiments demonstrate that mucosal hydrochlorothiazide not only inhibited the absorptive flux but also reduced the backflux. The reduction of the backflux of Cl was greater than the reduction of the Na backflux. Analysis of simultaneous determination of the backfluxes with the transepithelial conductance indicates that the sum of the partial ionic conductances for Na and Cl in the controlled state are greater than the transepithelial conductance. This finding indicates unambiguously the presence of electrically silent pathways for passive transfer of Na and/or Cl. In the tissues treated with hydrochlorothiazide the sum of the partial ionic conductances for Na and Cl were not different from the measured G_T.

The effect of metolazone, a chemically dissimilar member of the benzathiadiazide family of diuretics, was likewise examined. Metolazone inhibited (but not completely) net Na absorption. It also reduced I_{sc} and increased G_T. Like hydrochlorothiazide, it was effective only from the mucosal surface. In contrast to hydrochlorothiazide, in some tissues metolazone produced a transient stimulation of the short-circuit current concomitant with the increase in transepithelial conductance.

Table 2.--Effect of HCTZ on Simultaneously Determined Na and Cl Tracer Fluxes

	Mucosa-to-Serosa, n=8			Serosa-to-Mucosa, n=7		
	G_T (mS/cm^2)	J^{ms}_{Na} ($\mu M/cm^2 h$)	J^{ms}_{Cl}	G_T (mS/cm^2)	J^{sm}_{Na} ($\mu M/cm^2 h$)	J^{sm}_{Cl}
Control	0.669 ±0.147	1.70 ±0.25	2.45 ±0.30	0.346 ±0.035	0.52 ±0.07	1.34 ±0.12
HCTZ (0.1 mM)	0.951 ±0.186	0.40 ±0.05	0.69 ±0.24	0.665 ±0.115	0.23 ±0.02	0.29 ±0.04
p	<0.01	<0.005	<0.005	<0.02	<0.002	<0.001

p value represents significance between control and HCTZ treated group by paired analysis. Net fluxes are the differences between the mean unidirectional fluxes.

These data, when considered together with other information regarding NaCl absorption by the urinary bladder of the winter flounder, indicate that NaCl absorption occurs by an electrically neutral process probably involving an interdependent entry mechanism across the apical membrane. This process is relatively insensitive to loop diuretics but can be inhibited by thiazide-type diuretics. The reduction of the backflux of Na and Cl by mucosal hydrochlorothiazide indicates that a portion of this backflux component occurs transcellularly, perhaps through the same transport process by which absorption occurs. Although the mechanism for the increase in G_T is not clear, it might be secondary to an increase in cellular conductive pathways located on the apical membrane. This thiazide-sensitive NaCl transport system might be a model for NaCl absorption by the distal renal tubule. (Supported in part by NIH AM 25231.)

MORPHOLOGICAL EVIDENCE FOR IONOCYTES IN THE GILL EPITHELIUM OF THE HAGFISH, MYXINE GLUTINOSA L.

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The anatomy of the gill pouches of the Myxinoidea (Hofbauer, Biologia gen. 12:330, 1934; Rauther, Morph. Jb. 75:613, 1935) is fundamentally different from the arrangement of gill lamellae in all other aquatic vertebrates (Dunel and Laurent, in: Epithelial Transport in the Lower Vertebrates, B. Lahlou, ed.). The gill folds, which are irrigated with venous blood in a countercurrent manner to the perfusing water (Pohla et al., Zool. Scripta 6:331, 1977), are generally accepted to represent structures for gas exchange (Morris, J. Exp. Biol. 42:359, 1965). Electron microscopic analysis of gill epithelia in various species of euryhaline fish revealed different types of epithelia in relation to the adaptation to either the freshwater or the salt water habitat (Shirai and Utida, Z. Zellf. 103:247, 1970; Doyle and Epstein, Cytobiol. 6:58, 1972; Sardet et al., J. Cell Biol. 80:96, 1979); and physiological experiments demonstrated the importance of the chloride cells (Keys-Willmer cells) for ion-regulating processes. The present study was intended to decide whether similar cells are present in the epithelium of the gills of the hagfish.

Materials and Methods

Adult hagfish were obtained through the kindness of Dr. Foster, St. Andrews, Canada. After a few days of acclimation in a recirculating cooled seawater tank the animals were prepared for electron microscopy: The hagfish were exposed to MS 222 for ten minutes. A catheter was introduced into the ventral aorta and the vascular system was perfused with ice-cold physiological saline. The ice-cold fixation fluid (2% paraformaldehyde, 1% glutaraldehyde, 0.5% picric acid, 0.2 M cacodylate buffer adjusted to 980-1000 mOsm/l) was added continuously with constant perfusion pressure. After ten minutes the tissue was excised and fixed in the same mixture for an additional hour, rinsed in chilled buffer and postfixed in buffered 1% OsO_4. Tissue samples from all regions of the gill pouch were dehydrated and embedded in EPON 812. Thin sections were stained with uranyl acetate and lead citrate.