

and had previously been inhabited by crabs. The results given in Table 2 suggest that P. acadianus prefers Littorina covered with Hydractinia, where P. pubescens does not, although more complex factors than preference alone may be involved in nature.

The above data strongly suggests that within the area studied competition for Littorina shells must be most intense between P. pubescens of all size categories and smaller size P. acadianus. However, differential shell selection based on the presence of Hydractinia may help to reduce competition and thus niche overlap at this level. Presumably, larger individuals of P. acadianus face no interspecific competition in selection of shells. Availability of shell type may be an important factor in nature. This is suggested by the higher percentage of P. pubescens which chose Thais shells in the laboratory than would have been predicted from the field samples.

Table 2

SELECTION BY CRABS OF SHELLS WITH AND WITHOUT Hydractinia COLONIES

	<u>Littorina</u> with <u>Hydractinia</u>	<u>Littorina</u> without <u>Hydractinia</u>	Chi square df = 1
<u>P. acadianus</u>	Observed: 29	10	p < .01
<u>P. pubescens</u>	Observed: 10	30	p < .01

Some preliminary observations of intraspecific agonistic behavior in P. pubescens were made. One hundred ninety-four separate encounters between crabs of the same carapace length were analyzed with a multi-channel operations recorder. As reported by Hazlett and Bossert (Anim. Behav. 13:357-73) for other pagurid species, cheliped extension followed by retreat were the display complexes most frequently observed.

This work was supported by NSF Grant No. GY-7493, and Williams College research funds.

1970 #25

ORGAN CULTURE OF KIDNEY TUBULES OF FLOUNDER (Pseudopleuronectes americanus)

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Dissected and incubated tubular masses of the flounder kidney have been widely used for the study of renal organic acid transport. We have used the preparation to study protein transport and metabolism by kidney tubular cells (Maack and Kinter, Am. J. Physiol. 216:1034, 1969). In order to obtain more information on the intracellular pathways of protein transport I developed a long-lasting in vitro preparation of flounder renal tubules. In this report I describe an organ culture preparation in which the renal tubular cells remained viable for at least three weeks of culture.

We have previously reported (Kinter and Maack, Bull. MDIBL 7:26, 1967) that dissected tubular masses of the flounder kidney were able to maintain their viability for up to 36 hours

when incubated in Forster's balanced saline medium at 14°C. Townsley and Scott (J. Fish. Res. Bd. Canada 20:243, 1963) reported that systaltic action of muscular cells, lining the tubular epithelium, was present for up to one month when the tubular masses were incubated in Medium 199 at 5°C; however, they did not test the viability of the tubular cells. In the present study, a modification of the culture conditions employed by Townsley and Scott was used and the viability of the tubular cells was tested by the capacity of the tubules to concentrate chlorophenol red in the luminal fluid, a process which requires expenditure of cell energy.

Flounder kidneys were removed from decapitated animals. The caudal kidney was teased in ice-cold Forster's saline medium and about 10 explants, 2 to 3 mg in weight each, were transferred to small plastic culture flasks containing 2 ml of the culture medium. The latter consisted of Medium 199 Hanks base with L-glutamine and with or without 10% fish serum added. NaHCO₃ was added to give concentrations up to 10 mM and pH's from 7.2 to 7.8. Penicillin, 100 U/ml and in some cases, streptomycin, 10 µg/ml were also added. The culture flasks were kept at 4°C in a horizontal position so that only a thin film of medium covered the tubular masses. The medium was changed every three days at which time one explant of each culture flask was dissected further and transferred to Forster's saline medium containing 2.5 x 10⁻⁵ M chlorophenol red. Under the microscope and at room temperature dye uptake was followed for one hour and compared to the dye uptake of freshly dissected tubular masses of a control kidney.

Maintenance of near normal rates of dye uptake for three weeks required the following conditions: (1) incubation temperature, 4°C, (2) pH of medium above 7.6, (3) 10% fish serum added to incubation medium, (4) penicillin was necessary to suppress contamination but streptomycin had deleterious effect upon the culture. Flounder tubular cells remained viable for up to 10 days when incubated in Forster's medium alone at 4°C. After the third week dye uptake by the tubular cells diminished but did not disappear for up to one month of culture. Mitotic figures were not detected in the preparations. Systaltic muscular action was observed occasionally.

This preparation should be useful in studies of intracellular phenomena during protein uptake as well as in *in vitro* studies of induction phenomena of organic acid transport.

Supported by USPHS Grant AM14241.

1970 #26

WATER ABSORPTION BY ISOLATED EEL INTESTINE DURING SEA WATER ADAPTATION

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Sea water adaptation by the eel is associated with an increase in sodium, chloride and water absorption by the intestine (Bull. MDIBL 9:23-26, 1969) which appears to be controlled by cortisol (Gen. and Comp. Endocrinol. 11:373-80, 1968) via the pituitary. An increase in water absorption by *in vitro* eel intestine can also be elicited in fresh water adapted eels by daily injection of cortisol for 2 weeks prior to the study. The increase in water absorption during sea water adaptation appears to be sensitive to ouabain (Bull. MDIBL 9:23-26, 1969). However ouabain does not completely inhibit water absorption in either fresh or sea water adapted eels. The purpose of the present investigation was to determine the effect of ouabain and diamox on water absorption *in vitro* by intestines from freshwater adapted, 3 days in sea water and sea water