In the rat, BSP was localized to two prominent fraction, Y and Z, which results from a specific Y and a specific Z protein (J. Biol. Chem. 1969 (submitted)). The dogfish pattern exhibited neither a Y nor a Z peak. Dogfish liver supernatant did not inhibit Y or Z dye binding by rat liver supernates. With rat liver, the dye binding method detected Y and Z when 10% as much liver was used as in the studies with fish liver.

Comparative evaluation of Y and Z in all animals studied is presented in Table 1. In Table 1 + denotes a prominent Y or Z peak as illustrated by the elution pattern in the rat; - indicates the absence of a Y or Z peak as illustrated by the dogfish elution pattern, and ± signifies a trace of BSP binding in the Y or Z region but no clearly defined peak. In elasmobranch and teleost fish, as well as the gill-breathing amphibian mudpuppy, Y was not detectable and Z was either not detectable or present in trace amount. In liver from tadpoles of R. catesbiana, R. clamitans, and R. heckscherii prior to hind leg emergence, Y and Z were either not detectable or present in trace amounts. Following hind leg emergence and prior to complete tail resorption, tadpoles of each species had prominent Z and trace or nondetectable Y. Liver from adult frogs of each species had prominent Y and Z peaks. All lung breathing amphibians, reptiles, birds and mammals studied had prominent Y and Z binding proteins.

Values of t 1/2 for the plasma disappearance of intravenously administered BSP are also shown in Table 1. In mammals and birds, t 1/2 ranged from 2.7 to 11.1 min. The effects of temperature, blood flow and plasma proteins were not systematically studied.

Twenty minutes after injection of BSP, 68-88% of the injected dose was recovered in the liver and bile from rats, guinea pigs, goat, sheep and chicken, whereas only 2-8% of the administered dose was recovered in liver and bile from dogfish and mackerel.

It has been assumed that fish gills, and/or skin, are permeable to lipid soluble compounds including organic anions, although few data are available on the subject. If this thesis is correct, in the evolutionary transition from aquatic to terrestrial life, certain species replaced gills with lungs and the liver assumed an important role in the metabolism and excretion of various nonpolar organic anions such as drugs, dyes and steroids. In the present study, phylogenetic and ontogenetic appearance of Y and Z coincided with the transition from water to land, as well as with development of mechanisms for selective hepatic uptake of BSP. Similar ontogenetic development of Y, the major mammalian organic anion binding protein, has been demonstrated in guinea pigs and monkey (The Lancet 2:139, 1969; N. Eng. J. Med., 1970 (in press).

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1970 #24

ASPECTS OF NICHE DIVERSIFICATION IN TWO SPECIES OF HERMIT CRABS

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Shell selection by hermit crabs can be considered a specialized case of habitat selection (Reese, E. S., Anim. Behav. 10:374-360). Studies of two sublittoral, sympatric species of hermit crabs (Pagurus pubescens and P. acadianus were conducted in an attempt to define their respective niches in terms of this major parameter.

Several hundred crabs were collected at Bar Harbor, Maine on two occasions in August 1970 and the distribution of shell types inhabited by both species were recorded as well as crab hts for P. pubescens (Table 1). Weights for *P. acadianus* are from Grant (Ecology 44:767-71) who reported somewhat similar data for *P. acadianus* collected in the Salisbury Cove area.

Table 1

DISTRIBUTION OF SHELL TYPE FROM FIELD COLLECTIONS

Species of Shell	*P. acadianus*			*P. pubescens*			
	N	Percent distribution	Mean weight in grams*	N	Percent distribution	Mean weight in grams	
Littorina littorea	52†	23.6	2.93 ± 1.2	207	81.2	1.33 ± 0.6	
							$p < .005$
Buccinum undatum	151	68.6	7.45 ± 3.5	26	10.2	2.6 ± 0.7	$p < 0.05$
							$p < 0.01$
Thais lapillus	9	4.1	2.67 ± 0.9	21	8.2	1.8 ± 0.7	

*Data from Grant, 1963.

†20 covered with *Hydractinia echinata*.

These data indicate that although there is some overlap in the species of shells inhabited by the two hermit crab species, *P. acadianus* was most frequently found in *Buccinum* shells while *P. pubescens* was usually found in *Littorina* shells.

It is possible that shell preference may be dependent upon crab size. The mean shell weight to crab weight ratios for crabs found in *Littorina*, *Buccinum* and *Thais* were 2.44, 1.22 and 1.23 respectively. The difference between the ratios for crabs in *Littorina* and *Buccinum* was highly significant ($p < .0005$) as was the difference for crabs in *Thais* and *Littorina* ($p < .005$). Noting the mean weight of P. pubescens found in Buccinum, it is evident that the largest crabs are found in shells with the highest volume/weight ratio. It remains to be seen whether this occurrence is due to a preference for a low shell weight/crab weight ratio in larger *P. pubescens* or to a limited availability of shells with a volume large enough for the crabs regardless of shell weight. There was a smaller difference between mean weights of the two crab species found in *Littorina* and *Thais* shells than in *Buccinum*. It is clear that *P. acadianus* are found in relatively larger shells than *P. pubescens* since crab weight is directly related to shell weight within any one species of shell.

To test for possible preferences in *P. pubescens*, a shell selection experiment was conducted in which crabs were removed from *Littorina* shells and each was given a choice of three shells (one *Buccinum*, one *Thais*, and one *Littorina*) of the same weight as their home shell (± 0.25 g). The results showed that preferences for *Littorina* were 67.5%, *Thais* 29.7%, *Buccinum* 2.7% of 37 trial runs. The differences between the three shell species selected were highly significant ($p < .001$), assuming the null hypothesis that crabs have no preference.

Thirty-eight per cent of the *P. acadianus* found in *Littorina* were in shells covered with *Hydractinia echinata* while none of the 207 shells with *P. pubescens* was covered by the hydrozoan. The possibility that a difference in preference exists was tested. Crabs of both species were removed from their home shells and presented with two novel *Littorina* shells, one covered with *Hydractina* and one bare. In each test the shells weighed within 0.1 g of one another and had previously been inhabited by crabs. The results given in Table 2 suggest that P. acadianus prefers Littorina covered with Hydractina, 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 Hydractina 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

	Littorina with Hydractinia	Littorina without Hydractinia	Chi square df = 1
P. acadiamus	Observed: 29	10	$p < .01$
P. pubescens	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.

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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