TABLE 2

RATE CONSTANTS (k_{in}) FOR ACCESSION OF IONS FROM PLASMA TO CRANIAL FLUID AND PERILYMPH

	Na^+	$K^+(RB^+)$	Cl^-	HCO_3^- (total CO_2)
			Hr^{-1}	
A. NORMAL				
Cranial	0.13 (n=4)	0.09 (1)	0.08 (4)	0.52 (2)
Perilymph	0.09 (1)	0.15 (1)	0.13 (2)	0.12 (3)
B. CARBONIC ANHYDRASE INHIBITED*				
Cranial	0.07 (2)	-	0.08 (1)	0.19 (3)
Perilymph	0.05 (1)	-	0.12 (2)	0.15 (3)

*80 mg/kg acetazolamide 24 hours before injection of isotope.

Table 2A gives the accession rate constants of the ions to these fluids, measured in the same way as previously described for CSF *(vide supra)* and for endolymph (Addink, *et al.* This Bulletin, 12, 1972). Despite the small number of samples, the data permit the suggestion that the movement of the two fluids may be in part controlled by different forces, with the cranial moving more rapidly and with a much higher turnover of HCO_3^-. Table 2B supports this idea in suggesting a decrease in the Na^+ and HCO_3^- accession during carbonic anhydrase inhibition.

The function, origin, and flow pattern of cranial (or extra-dural) fluid, which is found in considerable volume between the brain and skull of many elasmobranch and teleost fish, are entirely unknown. The perilymph is equally unknown, but cannot be ignored in otic physiology. This report is designed to indicate future possibilities in the study of these important substances.

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THE DISTRIBUTION OF L-ASPARAGINE SYNTHETASE IN THE ORGANS OF VARIOUS MARINE ANIMALS

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L-Asparagine synthetase catalyzes the formation of L-asparagine from L-aspartic acid and L-glutamine in the presence of ATP and magnesium ions. Conversely the oncolytic enzyme L-asparaginase

(E.C. 3511) decomposes L-asparagine to L-aspartic acid and ammonia. In tumors resistant to therapy with L-asparaginase, L-asparagine synthetase is present in concentrations exceeding those found in sensitive tumors (Biochem. and Biophys. Res. Comm. 31, 1, 1968). This finding is felt to explain the resistant state (Science 160, 533-535, 1968). In contradistinction to resistant tumors the normal organs of most mammals contain comparatively low levels of L-asparagine synthetase. The most salient exception to this generalization is that we have found the pancreas synthesizes L-asparagine at a vigorous rate. Presumably the L-asparagine so synthesized is used in turn for protein synthesis. In an attempt to explore the phylogenetic origins of this endowment the L-asparagine synthetase of the pancreas and other representative organs of a number of marine animals has been examined.

Organ homogenates (1:10, w:v) of freshly-excised organs (Table 1) were prepared in 0.1M Tris-HCl, pH 7.6 containing 1mM dithiothreitol and 0.5mM EDTA. The supernatant (12,000 xg) from the homogenate was assayed in triplicate for L-asparagine synthetase by a radiometric technique to be published elsewhere. Briefly 5 μl of supernatant were incubated at 37° for 30 minutes with 5 μl of a 'cocktail' composed of 0.02M L-glutamine and 0.02M ATP in 0.05M Tris-HCl, pH 7.6 containing 0.124 μC of 4-(^{14}C) L-aspartic acid. During this incubation period 4-(^{14}C) L-asparagine was synthesized. Subsequent steps involved enzymatic removal of unreacted 4-(^{14}C) L-aspartic acid, recovery, and counting of 4-(^{14}C) L-asparagine in a suitable scintillant containing Cabosil.

The results of these studies are given in Table 1. It can be seen that in the mouse *Mus musculus* and in the dog *Canis familiaris*, L-asparagine synthetase is present at highest specific activity in the pancreas, considerably lower levels of the enzyme are observed in the other organs surveyed. By contrast in the fish studied the levels of synthetase are low in every organ including pancreas whereas in the lobster *Homarus americanus*, a phylogenetically lower species, the enzyme is concentrated in the hepatopancreas. Both avian species studied (sea gull *Larus argentaus* and duck *Anas boschas*) exhibited comparatively high activities of the enzyme in the pancreas.

In view of the low activity of the synthetase detected in fish, L-asparaginase levels were measured (Cancer Res. 30, 929-935, 1970) in the organ homogenates of two of the species, the dogfish *Squalus acanthias* and skate *Raja ocellata*. There is precedent for the presence of L-asparaginase in the organs of cold-blooded animals (Acta Physiol. Scand. 8, 342-347, 1944; Arch. Internat. Phys. Bioch. 19, 369-398, 1922). The results of the present survey are given in Table 2. No L-asparaginase could be detected in the organs of the dogfish while the skate had the highest concentration of this enzyme in the pancreas, with varying lesser concentrations in other organs.

Pursuant to these observations, the L-asparagine pool was measured in these two species (Biochem. J., 1972 - in press). It can be seen (Table 2) that the free intracellular L-asparagine pool is large in the organs of the dogfish (especially in the pancreas, uterus, spleen, and kidney), but exceedingly small in the organs of the skate. For purpose of comparison, the levels of L-asparagine in selected organs of the mouse are given below:

	L-Asparagine Level	
Organ	nmol/g wet wt.	nmol/mg protein
Pancreas	381.1	3.43
Liver	173.0	2.08
Brain	95.3	1.66
Testes	83.9	1.05

TABLE 1

L-Asparagine Synthetase Activity in the Organs of Various Species

SPECIES	L-Asparagine Synthesized * (nmol/g/h)											
	PANCREAS	LIVER	SPLEEN	HEART	BRAIN	UTERUS	KIDNEY	SKELETAL MUSCLE	GILL	LUNG	MALE GONADS	INTESTINE
Dogfish, *Squalus acanthias*	<10.0	22.0	14.4	<10.0	<10.0	<10.0	15.1	<10.0	15.5	.....	20.7	<10.0
King of Norway, *Hemitripterus americanus*	15.3	14.3	<10.0	.....	10.5	<10.0	<10.0	<10.0	<10.0	.....	.....	<10.0
Eel, *Anguilla rostrata*	<10.0	16.3	31.0	.....	13.8	.....	11.7	<10.0	16.4	.....	<10.0	13.2
Lobster, *Homarus americanus*	106.6**	.....	.....	56.5	.....	.....	.....	58.0	58.6	.....	60.5	.....
Skate, *Raja ocellata*	<10.0	<10.0	23.3	<10.0	<10.0	<10.0	.....	<10.0	32.5	.....	.....	23.7
Sea Gull, *Larus argentaus*	349.1	11.2	84.2	.....	15.6	.....	24.2	13.8	.....	31.7	.....	.....
Duck, *Anas boschas*	542.2	<10.0	<10.0	<10.0	19.8	.....	<10.0	<10.0	.....	<10.0	.....	26.4
Mouse, *Mus musculus*	1723.0	<10.0	67.0	.....	30.0	.....	11.0	.....	.....	34.0	184.0	.....
Dog, *Canis familiaris*	882.0	168.0	72.0	.....	75.0	.....	51.0	.....	.....	61.0	.....	.....

* Mean values for 2-5 animals except only one value available for duck. The levels of L-asparagine synthetase (nmol/g/h) found were as follows: dogfish--spiral value, stomach and rectal gland, all <10; skate--spiral value = <10, stomach = 21.3, and rectal gland = 10.5; lobster--green gland = 53.8; duck--gizzard = 13.6 and shell gland = 33.2.

**Hepatopancreas

TABLE 2

L-Asparaginase and L-Asparagine Concentrations in the Dogfish, *Squalus acanthias* and Skate, *Raja ocellata*

	DOGFISH				SKATE			
	L-Asparaginase*		L-Asparagine		L-Asparaginase		L-Asparagine	
Organ	I.U./g	I.U./mg protein	nmol/g	nmol/mg protein	I.U./g	I.U./mg protein	nmol/g	nmol/mg protein
Spleen	.02	.001	406.0	20.30	.10	.001	70.0	0.94
Pancreas	0	0	779.4	19.48	.98	.023	2.2	0.05
Skeletal Muscle	0	0	56.7	1.13	0	0	0	0
Kidney	0	0	333.3	13.88	.17	.007	10.8	0.49
Uterus	0	0	663.2	30.14	.34	.011	11.4	0.42
Heart	0	0	237.0	15.80	0	0	0	0
Rectal Gland	0	0	27.5	1.96	--	--	--	--
Brain	0	0	57.5	2.21	.35	.010	12.2	0.38
Spiral Valve	0	0	170.2	5.67	.21	.007	64.4	2.38
Liver	0	0	62.8	1.57	.32	.004	11.0	0.14
Stomach	0	0	31.2	2.08	0	0	16.8	0.49
Gill	0	0	172.6	5.75	0	0	1.6	0.04

*50 μl aliquot of the organ homogenate was incubated for 2 hours with 1 ml of 0.01M L-asparagine in 0.05M Tris-HCl, pH 7.0 at 37°; the reaction was terminated with 100 μl of 1N HCl, and neutralized after 10 minutes with 100 μl of 1N NaOH. L-Aspartic acid was measured in an aliquot of the reaction mixture by an enzymatic spectrophotometric technique (Cancer Res. 30, 929-935, 1970). 1 I.U. of enzyme hydrolyses = 1 micromole of L-asparagine per minute at 37° and pH 7.0.

This finding points to the possibility that the dogfish, a species lacking L-asparagine synthetase, is able to obtain the amino acid by dietary means or to synthesize L-asparagine by some unusual route. Alternative routes of synthesis of L-asparagine from cyanide and L-serine or L-cysteine have been reported to occur in the vetch *Lathyrus sylvestris*, in seedlings of *Vicia sativa*, as well as in *Chromobacterium violaceum* (J. Am. Chem. Soc. 85, 2874, 1963). In the case of the skate, the low levels of cellular L-asparagine may be explained by the concomitant presence of L-asparaginase in the organs of that species. Future studies will focus on the concentration of L-asparagine in the proteins of fish and on the means by which fish lacking the conventional enzymemechanism for synthesizing L-asparagine regulate the cellular concentrations of this amide.