water aquaria at MDIBL. Egg capsules were collected from the side of the aquarium and opened with iris scissors. The eggs, which develop synchronously within a single capsule (30-300 eggs/capsule), were suspended in sea water and kept in plastic petri dishes. Eggs develop normally at temperatures below 25° C. For studies of polar lobe formation, solutions were either applied to eggs exogenously or were injected intracellularly by hydrostatic pressure with a Leitz micromanipulator and microinjection apparatus. For studies of glycosaminoglycan biosynthesis, normal and lobeless embryos (500-2,000 embryos/batch) which had developed for 6 days were incubated with $H_2{}^{35}SO_4$ and /or ^{3}H-glucosamine (100 μc/ml). To monitor structural protein biosynthesis embryos were incubated in presence or absence of β-aminopropionitrile (cross-linking inhibitor) with ^{14}C-proline or ^{14}C-lysine (10 μc/ml). All incubations were for 24 hours at room temperature (18° C). After labeling, embryos were separated from the sea water label solution, rinsed twice with sea water, transferred to 70% ethanol, and taken to dryness. The sea water labeling media, together with the first sea water rinse, were diluted to 80% ethanol (glycosaminoglycans and structural proteins precipitate). Resulting precipitates were washed twice with 80% ethanol, then taken to dryness. Analysis of material in the embryo and sea water precipitates is in progress at Kansas State University.

Treatment of spherical eggs with the divalent cation ionophores, X537A (50 μg/ml) or A23187 (20 μg/ml) in the presence of sea water supplemented with additional Ca (2-10X that in sea water) caused higher percentages of eggs to form polar lobes quickly than did ionophore or additional Ca alone. Solutions of $LaCl_3$ (5×10^{-2} - 10^{-7} M), in the absence of phosphate and bicarbonate ions, caused no inhibition of polar lobe formation at pH 6.5 At pH 7.3-7.7, however, polar lobe formation could be inhibited by La (5×10^{-3} M). Microinjection of spherical eggs with solutions of $CaCl_2$ ($0.34\text{-}10^{-4}$ M) caused very rapid formation of polar lobes. Injection of $MgCl_2$ ($0.5\text{-}10^{-2}$ M) also caused lobes to form. Higher concentrations of Mg were required than for Ca, and the lobes took longer to form. Injection of KCl ($0.53\text{-}10^{-4}$ M) caused few round eggs to form polar lobes; however, when injected into eggs which had already formed a normal polar lobe, a majority of eggs resorbed the lobe very quickly. Such results were predicted on the basis of earlier work (Dev. Biol., 37, 280-294, 1974). Injection of sea water, distilled water, or mineral oil neither caused round eggs to form lobes nor caused eggs with lobes to resorb them. Exogenous solutions of dibutyryl cAMP ($5 \times 10^{-3}\text{-}10^{-7}$ M) had no morphological effect on eggs. Although exogenous solutions of cAMP alone ($2.5 \times 10^{-2}\text{-}10^{-7}$ M) also had no morphological effect on eggs, when such solutions were injected (10^{-5}, 10^{-7} M), a majority of round eggs quickly formed polar lobes. Cyclic GMP, when applied exogenously, ($10^{-3}\text{-}10^{-6}$ M), produced no morphological effect on eggs. Preliminary injection experiments with cGMP (10^{-2}, 10^{-5} M), however, suggested that a significant number of round eggs formed polar lobes as a result of injection.

The results above are consistent with a possible role for intracellular Ca in control of cell shape. (Supported by grant HD07193 from the National Institutes of Health).

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An Investigation of Orotic Acid as a Precursor of L-Asparagine in Dogfish (*Squalus acanthias*) and Skate (*Raja ocellata*)

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The body fluids and proteins of fish contain L-asparagine but the biochemical route for the sysnthesis of this amino acid amide is unknown (MDIBL Bulletin 12: 68, 1972; ibid 13: 27, 1973). Several possibilities for the acquisition or manufacture of L-asparagine can be considered: 1) L-asparagine is assimilated via the diet; 2) L-asparagine is present in seawater and is taken up via the gills; 3) In vivo, L-aspartyl tRNA is amidated to yield L-asparaginyl tRNA which is utilized for protein synthesis with proteolysis then contributing L-asparagine to body fluids; 4) there is a recondite precursor for the carbon skeleton of L-asparagine. It is to the fourth possibility that the present experiments address themselves. A survey of possible intermediary metabolites was made, specifically of those molecules in which the carbon skeleton of L-asparagine was latent. Of these, orotic acid (Figure 1) was selected for detailed study inasmuch

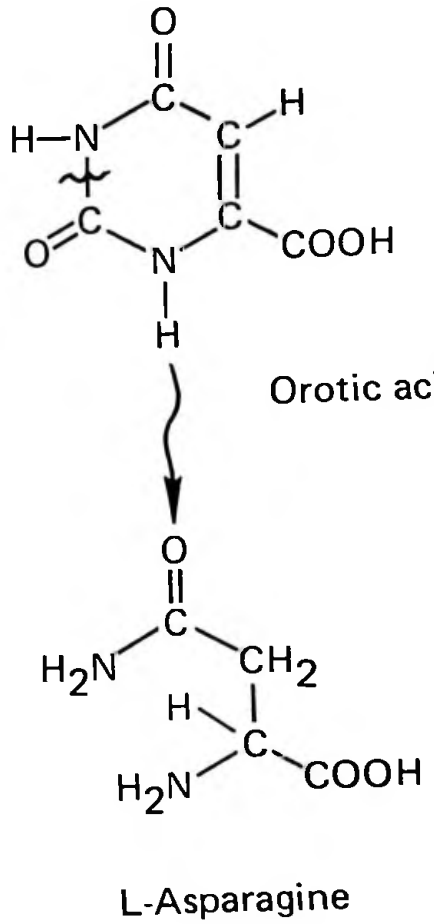

Figure 1

as this molecule contains the entire carbon and nitrogen sequence of L-asparagine. Dogfish were administered a large radioactive bolus (100 μCi, 49.1 mCi/mmole) of [6-^{14}C] orotic acid intravenously followed by measurement of the concen-

Table 1

The Incorporation of [^{14}C] Orotic into Amino Acids and Macromolecules in the Dogfish, *Squalus Acanthias, In Vivo*

	Incorporation into 4 Carbon Dicarboxylic Amino Acids (pCi/g or ml)				Incorporation into Macromolecules (pCi/g or ml)	
	L-Aspartic Acid		L-Asparagine			
Tissue	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr
Plasma	<280.0	<280.0	<260.0	<260.0	2476.6	30231.6
Erythrocytes	---	---	---	---	17121.0	16506.5
CSF	---	---	---	---	<430.0	<430.0
Brain	<280.0	<280.0	<260.0	<260.0	725.4	<430.0
Bile	---	---	<260.0	<260.0	<430.0	<430.0
Liver	<280.0	<284.4	<260.0	<260.0	548.7	4464.0
Pancreas	<280.0	<280.0	<260.0	<260.0	2055.3	1683.3
Spleen	<280.0	<280.0	<260.0	<260.0	1227.6	2352.9
Rectal Gland	<280.0	<280.0	<260.0	<260.0	9476.7	5412.6
Kidney	3544.5	1263.5	519.6	<260.0	1776.1	36297.9
Duodenum	---	<280.0	---	<260.0	948.6	1971.6
Heart	<280.0	<280.0	<260.0	<260.0	2771.4	1618.2
Muscle	<280.0	<280.0	<260.0	<260.0	<430.0	<430.0
Gill	<280.0	<280.0	<260.0	<260.0	14889.3	4398.9
Gastric mucosa	<280.0	<280.0	<260.0	<260.0	1255.5	827.7
Intestinal mucosa	722.0	---	<260.0	---	2325.0	<430.0
Testes	317.2	<280.0	<260.0	<260.0	<430.0	781.2

tration of free L-[4-^{14}C] aspartic acid and L[4-^{14}C] asparagine in the body fluids and proteins of representative viscera. For this purpose, a highly sensitive and selective enzymic radiometric assay was utilized (MDIBL Bulletin 12: 68, 1972). From the data presented in Table 1, it can be appreciated that considerable net radioactivity from [6-^{14}C] orotic acid persists in the viscera of the dogfish after intravenous administration of the precursor. This very likely represents incorporation of label into nucleic acids. However, the case with the dicarboxylic amino acids was quite different: L-[4-^{14}C] aspartic acid was found in relative abundance only in kidney; free L-[4-^{14}C] asparagine also was present only in that organ. In pronase-hydolyzed proteins from the principal organs of the dogfish, no significant labélling of L-aspartyl and L-asparginyl residues was observed. Moreover, high speed (12,000 g) supernatants of dogfish and skate kidney failed to degrade [6-^{14}C] orotate to L-[4-^{14}C] aspartic acid or L-[4-^{14}C] asparagine in vitro. These findings warrant the conclusion that while orotic acid can be degraded to L-aspartic acid or to L-asparagine in *Squalus acanthias*, quantitatively the route appears to be of minor importance.

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Inhibition of Mammalian L-Asparagine Sysnthetase by Material(s) Present in Homogenates of Fish Tissues: Possible Role of Mineral Cations.

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Thus far, attempts to detect mammalian-type L-asparagine synthetase (L-glutamine hydrolyzing, EC 6.3.5.4) in the organs of the dogfish, (*Squalus acanthias*) and skate (*Raja ocellata*) have been unsuccessful (MDIBL Bull. 12: 68, 1972; ibid 13: 27, 1973). Likewise, th enzymes for the condensation of cyanide and L-cyste to yield the L-asparagine precursor, -cyano-L-alan appear to be absent from these marine organisms (MDI Bull. 13: 27, 1973). Nevertheless, L-asparagine abundant in the body fluids and proteins of *Squalus* mo notably in the pancreas (19.48 nmole/mg protein) a spleen (20.30 nmole/mg protein). To resolve th apparent discrepancy, we hypothesized that mater present in the homogenates so far examined might b capable of depressing and even masking the biosynthe of L-asparagine. Several such inhibitors are known to b present in the tissues of mammals. These fall into thre classes; 1) low molecular or metallic inhibitors; 2 intermediary metabolites, and 3) large molecular o polymeric inhibitors. Calcium ions are the principal repre sentatives of the first catagory, and L-asparagine an S-adenosyl-L-methionine of the second catagory, an L-asparaginase, proteases and hyaluronic acid of the thir catagory. In previous communication (MDIBL Bull. 1 68, 1972) we have demonstrated that most organs of th skate, with the exception of plasma and muscl contained a considerable concentration of one of thes so-called inhibitors — [i.e., L-asparaginase (EC 3.5.1.1) It is the purpose of the present report to document th existence of other such endrogenous inhbitors in th tissues of these two species of fish.

Dogfish and skate were caught off the Maine coa and were killed by exsanguination. Twenty percent (w/ homogenates of the principal viscera were prepared 50% (v/v) ethanol or in 0.05 M Tris HCL buffer, pH 8. The homogenates were centrifufed at 12000 g for minutes and a 5 l aliquot of the resultant supernata was added to the reaction mixture previously describe