TABLE 2

Aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin deethylase (7-ERF) activities in hepatic microsomes from untreated winter flounder (*Pseudopleuronectes americanus*)

	AHH Activity (units/min/mg protein)		7-ERF Activity (pmol/min/mg protein)
	without α-NF	with α-NF (10^{-4} M)	
Group A[1]	0.16 ± 0.14 (2)[2]	0.66 ± 0.66 (2)	16.5 ± 14.8 (2)
	(0.05 - 0.26)[3]	(0.19 - 1.12)	(6 - 27)
Group B[4]	1.82 ± 0.98 (11)	0.95 ± 0.55 (11)	137 ± 57 (6)
	(0.28 - 3.15)	(0.19 - 1.63)	(64 - 230)

[1]Those fish where in vitro α-NF stimulated AHH activity; [2]Mean ± SD (N); [3]Range; [4]Those fish where in vitro α-NF inhibited AHH activity.

stimulated (Group A) or inhibited (Group B) hepatic microsomal AHH activity, with the implication that those fish in group B had partially induced MFO systems. The much higher ERF activities in the group B fish support this hypothesis, since ERF activity is preferentially induced by compounds that result in the formation of cytochrome P-448 (Burke and Mayer, Drug Metab. Disp. 2:583, 1974). The fact that 85% of the flounder tested had induced MFO systems prompted us to do similar experiments in dogfish and little skates. In these two species, caught in the same locale as the flounder, none of the untreated fish showed evidence of induction although hepatic microsomes from all DBA-pretreated dogfish and skates had elevated hepatic microsomal AHH activities which were inhibited by α-NF (data not shown).

Whether or not the induction of the hepatic MFO system of most of the winter flounder investigated here is due to exposure of these fish to environmental contaminants, such as the PAH, is still unknown. However, a recent observation made in our Florida laboratory where only 8 of 81 sheepshead (*Archosargus probatocephalus*) caught in the wild had induced hepatic MFO activities and where induction almost totally coincided with the spawning season (April and May) (Bend, Foureman, James, Second Internatl. Symp. Aquatic Poll. Pergamon, in press) suggests that normal physiological processes may also be related to induction of the hepatic MFO system in the flounder. (The flounder were not assayed during spawning season.) Obviously, more detailed investigations are required before induction of the MFO system in fish is routinely used as a biochemical index for chemical pollution of the aquatic environment.

ALANINE SYNTHESIS DURING STARVATION IN SKELETAL MUSCLE OF THE SPINY DOGFISH, *Squalus acanthias*

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The spiny dogfish, *Squalus acanthias* is able to survive for many weeks without food during which period it must rely on endogenous stores to provide both energy and materials for synthesis. The synthesis of urea must continue during starvation, since despite a significant loss of the nitrogenous product to the environment the urea content of plasma decreases only slightly (see below). A second synthesis of likely importance during starvation is that of glucose. In mammals, glucose or glycogen are the obligatory substrates for ATP generation in nervous and anaerobic tissues. It is unlikely that sufficient carbohydrate could be stored in the dogfish to satisfy the needs of prolonged starvation, so that gluconeogenesis is required. Both urea and glucose could be synthesized from a number of amino acids and the most likely source of these

is the hydrolysis of muscle protein. In mammals, it has been established that considerable metabolism of the amino acids thus arising occurs before they are released from the muscle into the blood (for transport to the sites of gluconeogenesis). The consequence of this metabolism is that just two amino acids, alanine and glutamine, comprise the major portion of the released amino acids (Felig et al. Science 167:1003, 1970). In elasmobranchs, however, glutamine release by muscle is low as is the concentration of this amino acid in plasma (see below).

In the present study, therefore, the magnitude of alanine release from skeletal muscle during starvation was measured, and factors affecting this release were investigated. Two approaches were used. First, in vivo measurements of amino acid release were made by comparing the amino acid contents of arterial and venous blood, respectively supplying and draining tail muscle. Second, the rate of in vitro alanine production was measured using isolated intact pelvic fin muscles. Both studies were performed on freshly caught (fed) dogfish and on dogfish maintained in captivity without food for various periods.

Male spiny dogfish, weighing at capture between 1.0 and 2.4 Kg, were used. They were caught on lines in Frenchman Bay, Maine and maintained in running seawater in a 3 X 5 meter tank. Water temperature in the tank varied between 12.5°C and 14.0°C. Blood samples were taken from the appropriate caudal vessels as follows: Fish were stunned by a blow on the head and immediately transected just posterior to the anus. Venous blood was withdrawn by inserting a polyethylene cannula and drawing blood into a heparinized syringe. Arterial blood was similarly withdrawn from the dorsal aorta in the anterior part of the fish (since insufficient blood remained in the caudal artery of the tail section). The blood (0.75 ml) was immediately centrifuged and samples of plasma were deproteinized with sulfosalicylic acid. The supernatant from deproteinization was dried under vacuum in the presence of a desiccant at room temperature and dispatched for amino acid analysis on an automatic amino acid analyzer (Durrum-500).

To follow changes in glucose and urea concentrations in plasma, blood samples were withdrawn from the caudal vessels of intact unanesthetized fish and centrifuged. Sodium fluoride (final concentration 0.01 M) was added immediately to plasma samples destined for glucose determination in order to inhibit glycolysis. Alanine biosynthesis was assayed in vitro using pelvic fin depressor muscles which were excised rapidly from anesthetized dogfish, weighed and transferred to flasks containing 5.0 ml dogfish Ringer's solution that had been previously gassed with 99% O_2 - 1% CO_2 and contained 3 mM each of glucose and amino acid. The flask was gassed briefly with the same O_2-CO_2 mixture, stoppered and incubated with shaking at 15°C for 90 min. After incubation the muscle was removed, blotted, freeze-clamped and powdered in a mortar surrounded by dry ice. The powdered muscle and an aliquot of the incubation medium were analyzed for alanine content as described by Goldstein and Newsholme (Biochem. J. 154:555, 1976). Glucose was assayed using glucose oxidase, peroxidase and o-dianisidine. Urea was assayed by a modification of the colorimetric method of Archibald (J. Biol. Chem. 157:507, 1945).

Spiny dogfish kept in captivity lost approximately 1% of their initial weight per day over the period studied (Table 1). Plasma urea concentration decreased progressively with time. In three fish studied in detail the urea concentration fell from an average of 358 µmoles/ml to an average of 330 µmoles/ml in 19 days, although this fall was not regular (Figure 1).

Since alanine is a likely source of plasma glucose during starvation, plasma glucose levels were measured after different periods of starvation. This parameter showed the greatest variation between individuals. The data in Table 1 show that there is no significant change in plasma glucose levels during starvation, although a range of 0.34-8.29 mM was observed. In an attempt to eliminate individual variation three fish were selected and samples were taken from the same fish at intervals over a period of nineteen days starvation. No consistent trend was observed.

Arterial-venous (A-V) differences in amino acid concentration offer the most direct means of estimating the rate of amino acid release from muscle. Amino acid analysis of plasma from blood going to and from

TABLE 1

Changes in body weight, proportional weight of liver and plasma glucose concentration during starvation

Days Starvation	Percent of Initial Body Weight Lost per Day	Liver Weight (% of body weight)	Plasma Glucose (μmoles/ml)
0-1		9.70 ± .34 (4)	3.38 ± .44 (11)
9-14	1.01 ± .10 (10)	9.40 ± 1.83 (4)	4.07 ± .47 (9)
19-25	0.98 ± .05 (12)	7.22 ± 1.10 (8)	3.59 ± .94 (8)
43	0.46 (1)	9.86 (1)	7.34 (1)

Values are means ± S.E. Number of fish per group is indicated in parentheses.

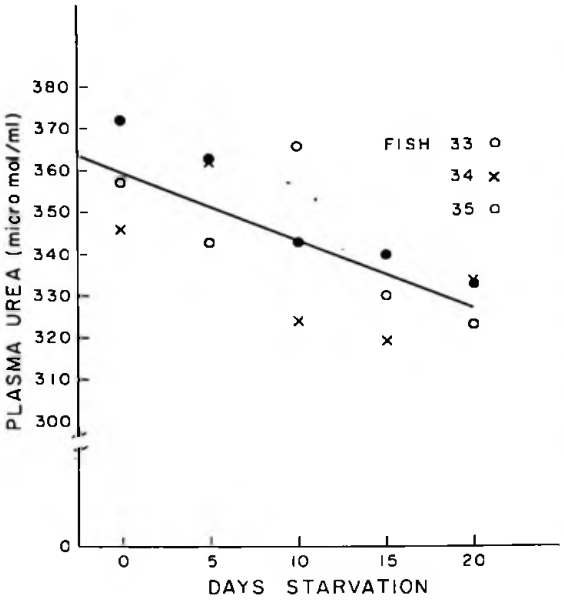

Fig. 1 Plasma urea concentrations in three starved dogfish.

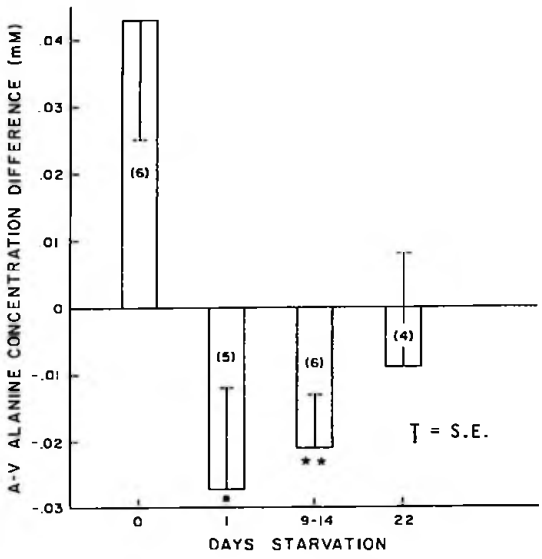

Fig. 2 Caudal arterio-venous concentration differences in starved dogfish.
* P < .05 ** P < .01

tail indicated that alanine was the only amino acid consistently being released from muscle in significant quantities. As shown in Figure 2, alanine was extracted from arterial plasma by muscle of freshly caught fish (0-day). However, by one day after capture alanine was released into circulating plasma and continued to be released (albeit in lesser amounts) by starving fish for at least 2 weeks.

Net production of alanine by skeletal muscle was also demonstrated in in vitro experiments in which pelvic fin muscles were incubated in Ringer's solution and changes in alanine content measured. As shown in Table 2, appreciable quantities of alanine were produced in the presence of 3 mM glucose and 3 mM isoleucine. This accumulation is greater when the muscles have come from starved animals (Table 2), although the difference was statistically significant only with muscles from fish starved for 2 weeks.

The ability to demonstrate alanine synthesis in vitro opened the way to an investigation of the conditions necessary for alanine production and of the metabolic route utilized. Omitting isoleucine from the

TABLE 2 ALANINE PRODUCTION BY PECTORAL FIN MUSCLES INCUBATED IN VITRO

Days Starvation	Number of Experiments	Alanine Content (μmoles/g)				
		In muscle after incubation	In medium after incubation	Total after incubation	In muscle*before incubation	Alanine produced
1	6	1.51±.29	2.71±.36	4.22±.53	1.93±.36	2.29±.31
12-17	6	0.99±.12	3.59±.55	4.58±.66	1.23±.09	3.35±.60
20-22	7	1.05±.07	3.67±.24**	4.72±.23	1.25±.11	3.47±.24**

Incubations were carried out for 90 minutes at 15°C in dogfish Ringer's solution supplemented with glucose (3mM) and isoleucine (3mM). Results are means ± S.E.

*obtained from an adjacent muscle, freeze-clamped before incubation **p<.05 vs. one-day starvation

incubation medium reduced alanine production by muscles from 14-day starved fish to 10.7 ± 4.2% (means ± S n = 4) of the amount produced in its presence. Omission of both isoleucine and glucose lead to the production of only 4.5 ± 4.7% (n = 4) of the alanine produced in their presence. The omission of glucose alc caused no significant change in the rate of alanine production. When leucine (3 mM) was substituted for isoleucine, no significant change in alanine production was observed. Substantially the same results were obtained using fish one day after capture (that is, 'fed' fish). It would appear that for alanine production, an exogenous amino acid is required but that glucose per se is not.

To determine whether the enzyme phosphoenolpyruvate carboxykinase (PEPCK) is involved in alanine synth sis in skeletal muscle, 3-mercaptopicolinic acid, an inhibitor of the enzyme, was tested. With muscles fr 17-20 day starved fish, the inhibitor (1 mM) did not reduce the rate of alanine synthesis significantly. This was so whether isoleucine or leucine was the added amino acid and whether or not glucose was present.

In summary, the above experiments demonstrate that skeletal muscle of the spiny dogfish can synthesize alanine from other amino acids. During starvation the alanine is released into the circulation where it can be transported to the liver and utilized for biosynthesis of urea and glucose. This process helps the fish to maintain blood urea and glucose levels during starvation. Nevertheless, the supply of this amino acid to the liver is insufficient to maintain urea biosynthesis at a normal level during starvation.

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TRANSPORT OF SODIUM INTO BRUSH BORDER MEMBRANE VESICLES ISOLATED FROM FLOUNDER INTESTINE AND FLOUNDER KIDNEY TUBULES

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In recent studies on the intact epithelium of the intestine from the winter flounder, *Pseudopleuronect americanus*, it was demonstrated that Cl^- absorption was tightly coupled to the transport of Na^+ (Field and Smith, Bull. MDIBL 15, 1975; Field, Bull. MDIBL 16, 1976). Experiments by Frizzell, Smith and Field (to Bull.) further demonstrated that sodium-coupled chloride entry at the luminal border of the flounder intestine was inhibited by a cAMP-mediated process and also by furosemide. These findings suggest that the Cl^--absorptive mechanism in the teleost intestine may be similar to that in rabbit ileum and gallbladder (Schultz and Frizzell, Biochemistry of Membrane Transport, Springer-Verlag, Neidelberg/New York, 1977).