maintenance pump (Tosteson and Hoffman, J. Gen. Physiol., 44: 169, 1960) should lead to disruption of cell volume as a consequence of the Gibbs-Donnan effect. Pump inhibition would also negate the supportive aspect of limiting net flux to only that ion whose net passive movement would favor volume restoration. Since cessation of ion pumping would further insult cell volume it would be expected that membrane permeability might undergo large increases in the presence of inhibitors of active transport. This is in fact the case as seen in the data for Na influx and K efflux associated with ouabain-treated cells. In light of the magnitude of the permeability changes it is not at all unlikely that contra-gradient flux should be increased to values equal to or in excess of values seen during ion pumping. In other words ouabain has affected the ion pumping of the cells but superimposed upon its effect is the volume regulating response of increased membrane permeability.

In summary the data show that flounder cells respond to volume perturbation by regulating volume back toward steady-state values. The water movements during the regulatory process are associated with movements of the inorganic cations Na and K down their respective electrochemical gradients. These passive ion movements are a consequence of changes in membrane permeability which are only partly selective to Na and K. Ion pumping plays a supportive role during the process of volume regulation but is not essential as shown by the data on ouabain-treated volume-perturbed cells. Yet this is not to say that these cells have no need of active ion pumping since it is the energy stored in the Na and K electrochemical gradients established and maintained by the Na-K pump that produces the driving force for ion and therefore water flux during volume regulation.

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1973 #9

THE ACTIVITIES OF HEPATIC MICROSOMAL ENZYME SYSTEMS IN FISH

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The activities of three hepatic microsomal enzymes were determined in winter flounder *Pseudopleuronectes americanus*, killifish *Fundulus heteroclitus*, the small skate *Raja erinacea*, and mackeral *Scomber scombrus*. Enzyme assays were performed on whole liver homogenates. Phosphorothioate detoxification was measured by the method of Neal and DuBois (J. Pharmacol. Exp. Therap. 148:185, 1965) using O-ethyl-O-(4-nitrophenyl)-phenyl phosphonothioate (EPN) as the substrate and O-demethylase activity was measured by the procedure of Netter and Seidel (J. Pharmacol. Exp. Therap. 146:61, 1964) using p-nitroanisol as the substrate. The N-demethylation of aminopyrine was measured by the method of La Du et al. (J. Biol. Chem. 214: 741, 1955) and the activity expressed as micrograms of 4-aminoantipyrine formed per 100 mg. of liver in 30 minutes. The activities of the EPN detoxification system and O-demethylase are expressed as micrograms of p-nitrophenol formed by 50 mg. of liver in 60 minutes from EPN and p-nitroanisol respectively.

Table 1 summarizes our studies on three enzyme activities in the four species of fish examined. These activities are quite low, approximately one-third those observed in mammals. Since pheno-

TABLE 1

HEPATIC MICROSOMAL ENZYME ACTIVITY IN FISH

SPECIES	ENZYME ACTIVITY		
	N-Demethylase	EPN Detoxification	O-Demethylase
	µg/100 mg liver/30 min.	µg/50 mg liver/hr.	µg/50 mg liver/hr.
Winter flounder	0.85 ± 0.17 (7)*	2.29 ± 0.22 (12)	3.24 ± 0.26 (12)
Killifish	0.75 ± 0.11 (5)	2.13 ± 0.23 (5)	3.70 ± 0.33 (5)
Small Skate	0.61 ± 0.08 (3)	2.48 ± 0.42 (3)	8.45 ± 0.97 (3)
Mackeral	1.17 ± 0.24 (4)	1.29 ± 0.20 (4)	1.73 ± 0.37 (4)

*Mean ± S.E., the number in parenthesis denotes the no. of fish

TABLE 2

HEPATIC MICROSOMAL ENZYME ACTIVITY IN FISH
PRETREATED WITH PHENOBARBITAL OR AROCLOR COMPOUNDS

SPECIES	PRETREATMENT*	ENZYME ACTIVITY		
		N-Demethylase	EPN Detoxification	O-Demethylase
		µg/100 mg liver/30 min.	µg/50 mg liver/hr.	µg/50 mg liver/hr.
Winter flounder	Phenobarbital	0.63 ± 0.06 (3)**	1.68 ± 0.11 (3)	3.10 ± 0.42 (3)
	Aroclor 1254	0.79 ± 0.17 (3)	1.84 ± 0.33 (3)	3.80 ± 0.11 (3)
	Aroclor 1260	0.59 ± 0.14 (5)	2.25 ± 0.21 (5)	3.90 ± 0.33 (5)
Killifish	Phenobarbital	0.92 ± 0.26 (4)	2.96 ± 0.49 (4)	4.13 ± 0.66 (4)

*Fish received drug, 50 mg/kg, i.p. for 5 days

**Mean ± S.E., the number in parenthesis denotes the no. of fish.

barbital and polychlorinate biphenyls (Arclor) have been shown to be potent inducers of hepatic microsomal enzyme activity in rats (Chen and DuBois, Toxicol. Appl. Pharmacol. 1973, in press) the activities of these enzymes were also determined in fish pretreated with either sodium phenobarbital, Aroclor 1254 or Aroclor 1260, 50 mg/kg i.p for 5 days. As shown in Table 2 no appreciable inducing effect by either sodium phenobarbital or PCB was found. However all fish receiving phenobarbital showed definite signs of sedation.

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1973 #10

INVESTIGATIONS OF THE BIOSYNTHESIS OF L-ASPARAGINE IN THE SKATE *Raja ocellata* AND DOGFISH *Squalus acanthias*

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In a previous communication we reported the apparent absence of L-glutamine-dependent L-asparagine synthetase in the organs of several fish (MDIBL Bull. 12, 68, 1972). At the same time extracellular L-asparagine in at least one of the species examined was abundant. To investigate the source of this L-asparagine we have introduced six analytical changes designed to enhance the likelihood of detecting L-asparagine synthetase in fish: 1) more concentrated homogenates were prepared; 2) the homogenates were dialyzed against 30 percent buffered glycerin to further concentrate the enzymes in them and to remove putative low-weight inhibitors; 3) whole homogenates and not simply supernatants were used for the analyses in the event that the synthetase was particulate (Biochem. Pharm. 21, 39, 1972); 4) in addition to L-glutamine ammonia was examined as precursor of the future amide of L-asparagine (J. Biol. Chem. 247, 6708, 1972); 5) the cyanogenic route to L-asparagine, which operates in many plants (J. Biol. Chem. 244, 2632, 1969) and in several species of bacteria (J. Am. Chem. Soc. 85, 2874, 1963), also was studied in these homogenates:

$$\text{L-cysteine} + \text{HCN} \longrightarrow \beta\text{-cyano-L-alanine}$$

$$\beta\text{-cyano-L-alanine} + H_2O \longrightarrow \text{L-asparagine;}$$

6) ammonium chloride was added to a final concentration of 0.1M in select cases with the intention of inhibiting the L-asparaginase known to be present in the body fluids of the fish used and of thereby preventing the artifactual hydrolysis of the product of the enzyme under study (Biochim. Biophys. Acta 185, 228, 1969).

Fish were killed by decapitation or exsanguination and their organs frozen at -20° until the time of analysis. L-asparagine synthetase was measured radiometrically as described previously (MDIBL Bull. 12, 68, 1972). In the present study for increased sensitivity L-asparaginase also was measured radiometrically using [U-^{14}C]-L-asparagine as substrate. The [U-^{14}C]-L-aspartic acid arising from the hydrolysis of the amide was β-decarboxylated essentially as described previously (J. Lab. Clin. Med. 81, 455, 1973). This technique is capable of detecting as little as 1×10^{-5} I.U./ml of L-asparaginase in a 5 μl sample. The lithium citrate system of Oreskes was used to separate