the intracellular sodium alone. After preparing Table 1 from several different bases, it was decided to present the data in a simple ratio: counts per gram of wet tissue/counts per milliliter of terminal plasma. Another method was counts per milliliter of tissue water/half-time counts per milliliter of plasma. One can calculate the ratios tissue water/plasma using Table 1, and Table 3 in the preceding report.

In a general way, these ratios can be taken as a measure of the rate at which the sodium spaces of the various tissues are filled. For brain and cartilage the ratios increase with time. For the rectal gland, the ratio remains constant with time. Except for a few out-of-line values the ratios form an orderly picture.

These ratios seem to reflect the intracellular sodium content. If the ratios merely measured extracellular space/plasma, one would expect that in eleven hours the ratios would approach one, which they do not do. Indeed if one takes the few reported intracellular concentrations for sodium in brain and muscle then the ratio mEq Na per ml tissue water/mEq sodium per ml plasma is nearly equal to the ratio of counts per ml of tissue water/counts per ml plasma. Of course certain tissues such as cartilage and skin have large non-cellular spaces. It does seem possible from the above data and that in the preceding report, to approximate the sodium content of various organs.

The ratios indicate that since the various tissues do not have a uniform rate of sodium exchange, it is not possible to define when the animal is in equilibrium with an injected dose. However, for practical purposes, two hours seems a generalized time for good internal mixing. Graphically, falling plasma curves begin to flatten out at this time, and the counts per ml plasma correspond to what one would expect if the total counts were mixed in a volume equal to 20% of the body weight.

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1967 #7

SODIUM EXCHANGE THROUGH THE EXTERNAL SURFACES OF Myxine

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Unlike marine elasmobranchs which produce a hypertonic plasma by adding to blood electrolyte osmotically active organic moieties, the marine cyclostomes effect a slight hypertonicity largely through electrolytes. It is of interest to know whether electrolyte exchange takes place through the skin and gills in all marine forms, and if so to estimate the rate of exchange. This statement carries no implication that there must be a directed flux.

Morris (J. Exp. Biol. 42:359-71, 1965) gives numerous data on the salt and water content of the hagfish, Myxine, together with data on urine flows, drinking sea water, etc. The study here is concerned solely with sodium exchange between the external surfaces of hagfish and its environment as measured with Na^{22}.

The handling of the fish (M. glutinosa) was essentially that described by Rall and Burger (Am. J. Physiol. 212:354-56, 1967). In addition, a cord was tied around the animal anterior to the cloaca to prevent fluid loss while the fish was mounted for sampling. In efflux studies this same technique was used prior to injection of the isotope. Some fish (A, D series) were used

immediately after capture (about 2 hrs), and some were saved and kept either in a laboratory aquarium at 12.5° C or in a refrigerated bath at 5.5° C. During the experiments, fish were placed in measured volumes of aerated sea water in plastic bags cooled externally by ice. Measured Na^{22} was injected or added to the sea water. When injected, the animals were rinsed in a preliminary bath before being placed in the final bath. The isotope was counted in a well-scintillation detector with a 1 ml or less sample.

Work with these beasts is frustrating. There are no methods available for serial sampling of blood, and the clouds of slime produced when the fish are disturbed makes sample-purity difficult.

The data are presented in Tables 1, 2, and 3. In Table 3, one notes that fish in captivity (B, C series) have an increased out → in exchange when compared to fresh fish (A series). A deterioration of plasma electrolyte has been noted before by us and others. The C series, Table 1,

Table 1

SODIUM INFLUXES FOR Myxine

Fish	Wt. gms	Hrs	Na^{22} counts/min/ml			
			Plasma	Urine	Bile	Intestinal fluid
A. Freshly caught fish						
A-1	196	1.5	221	248	10	-
A-2	214	2.5	359	-	-	-
A-3	147	3.5	621	542	108	-
Bath: 28,800 counts/min/ml; 2 liters. Temp. 8.5- 10 C. A-3: Cloaca ligated for last hour.						
B. Fish kept in laboratory for 8 days. Temp. 12.5 C.						
B-4	112	2.2	994	788	15	-
B-5	85	3.3	3,496	3,298	17	-
Bath: 24,000 counts/min/ml; 2 liters. Temp. 7.5- 10 C.						
C. Fish kept in laboratory for 4 days. C-4 lab aquaria, temp. 12.5 C; C-5,6 refrigerated 5.5 C.						
C-4	84	2.0	1,936	-	0	22,680
C-5	221	2.5	2,819	1,090	0	45
C-6	108	2.8	1,076	120	0	27,829
Bath: 39,900 counts/min/ml; 1.1 liters. Temp. 6.5 -7.8 C. Cloacae ligated before being placed in bath.						

show, as Morris found, that during a stated period some fish drink sea water and others do not. There is no evidence in this series that the sodium uptake is augmented by drinking (compare C-5 with C-4,6). There is a brisk exchange from blood to gut water (Table 2, D), and the possibility exists that there may be a unidirectional flux from blood to gut contents. The total flux data for the external surfaces, as distinct from gut, indicate the hagfish has no predominant flux in one direction.

Analysis of the urines in Tables 1 and 2 agree with Morris that the U/P sodium ratio is less

Table 2

SODIUM EFFLUXES FOR Myxine

Fish	Wt. gms	Hrs	Na^{22} counts/min/ml				
			Bath	Plasma	Urine	Bile	Intestinal fluid
D. Fresh fish							
D-1	205	1	74				
		2	114				
		3	134	60,182	36,310	3,537	3,638
D-2	177	1	80				
		2	126				
		3	159	104,760	62,080	4,040	1,358
D-3	168	1	174				
		2	202				
		3	233	89,892	41,800	2,205	11,392

Each fish received 4,554,400 counts/min subcutaneously. Rinsed and placed in 1 liter bath. Animals ligated anterior to cloaca before injection.

Table 3

CALCULATED SODIUM INFLUXES AND EFFLUXES FOR Myxine

A. Sodium Influx in Myxine

	meq Na/hr-100 g fish
Fresh fish A-1	0.076
A-2	0.070
A-3	0.082
	Av. 0.076
8 day fish B-4	0.26
B-5	0.52
	Av. 0.39
4 day fish C-4	0.21
C-5	0.20
C-6	0.09
	Av. 0.17

Values used for calculation: Sodium space 0.30 (derived from efflux below); plasma sodium, 510 meq/1; bath sodium, 440 meq/1.

B. Sodium Efflux in Myxine

Fresh fish	meq Na/hr-100 g fish		Na space
	Hours 1-3	Hours 2-3	
D-1	0.13	0.08	0.36
D-2	0.16	0.10	0.29
D-3	0.10	0.10	0.30
Averages	0.13	0.093	0.32

than one in most animals. Fish A-3 was stripped of urine, then ligated anterior to the cloaca, so it is fairly safe to say the urine collected was made during the experimental period. While many fish apparently had residual urines which diluted incoming urine, there are enough urines close to U/P ratios of one to warrant agreement with Morris's average sodium U/P of 0.94. Morris calculated that the urinary loss of sodium ranged from 0.002-0.12 mEq/100 g-hr. This is the order of magnitude of the external fluxes.

While our data does not demonstrate the source of sodium uptake, it does not seem to be from the skin or gills.

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1967 #8

PROTEIN SYNTHESIS IN EMBRYOS OF Fundulus heteroclitus

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The effect of cyanide and Actinomycin D on amino acid incorporation into proteins of embryos of Fundulus heteroclitus was found by these investigators to be dependent upon the stage of development and the amino acid used (Bull. M.D.I.B.L. 6:9-11, 1967 and Hahnemann Symposium, "Epithelial-Mesenchymal Interactions," to be published). These observations opened the possibility of investigating the role of both bioenergetics and RNA synthesis in the control and regulation of specific protein synthesis during embryonic development. Therefore, further studies were conducted to verify and extend these findings.

The methods of culturing Fundulus embryos were the same as reported previously (Exptl. Cell Res. 44:471-88, 1966). Amino acid incorporation into protein was defined as the amount of radioactivity in hot trichloroacetic acid-insoluble material extracted from embryos which had been incubated for two hours in the presence of C^{14}-labeled amino acids. When inhibitors were used, they were present in the incubation medium 30 minutes prior to the introduction of the labeled amino acid.

The stimulation of lysine incorporation by both cyanide and Actinomycin D was found to be greatest immediately following fertilization. The effect diminished considerably by the time of first cleavage and after the four-cell stage was reached both agents inhibited lysine incorporation. This presumed stimulation of synthesis of protein rich in lysine by cyanide and Actinomycin D was not observed using other amino acids (leucine, phenylalanine, valine, algal amino acid mixture). It would appear that for a short time immediately following fertilization a particular protein synthesis occurs without need of aerobic metabolism or RNA synthesis; indeed, its rate of synthesis is enhanced by the prevention of aerobic energy flow and new RNA synthesis. The protein species involved deserves much further study.

Other conditions useful for the experimental control of protein synthesis in Fundulus embryos were studied. These experiments were performed on embryos of late stage which very actively incorporated lysine. The substance cycloheximide, an inhibitor of protein synthesis in some systems, had no effect on amino acid incorporation up to concentrations of 20 μg/ml. Pactamycin (20 μg/ml) inhibited lysine incorporation approximately 85%. Puromycin, up to 100 μg/ml, inhibited the system approximately 60%.