ESTIMATION OF EXTRACELLULAR SPACE AND INTRACELLULAR ION CONCENTRATIONS IN OSMOCONFORMERS, HYPO- AND HYPEROSMOREGULATORS

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The process of adjustment by fishes to ionic or osmotic changes in their surrounding medium involves mechanisms functioning at the cellular as well as organ level. This is true for two reasons. First organ systems are frequently unable to cope with large external variations in sufficient time to prevent changes in their internal medium. Second even after the process of acclimatization is complete at the organ level the extracellular fluid concentrations are frequently maintained at a new steady-state. Most research on osmoregulation in fishes has concentrated on the function of organ systems in regulating internal concentrations. However as Lutz (Comp. Biochem. Physiol. 41A: 77-88, 1972) has recently pointed out osmotic and ionic adjustments by individual cells are at least as important as adjustments made at the organ level.

The primary obstacle to examination of intracellular osmotic and ionic concentrations has been the inadequacy of extracellular space determinations. Several of the most acceptable values for extracellular space (ECS) have been obtained with inulin, ^{35}S labelled SO_4, Cl^- and mannitol. Of these inulin, given intravenously, seems to have found the widest acceptance. This report deals with the relative effectiveness of radioactively labelled inulin and polyethylene glycol (PEG) in determining extracellular space and measurements of intracellular concentrations in various fishes.

American eels *Anguilla rostrata* weighing 75 - 200 grams, were acclimated for at least two weeks in either flowing seawater or flowing freshwater prior to use. Winter flounder *Pseudopleuronectes americanus* weighing 80 - 150 grams, were maintained in flowing seawater two weeks prior to use. Dogfish *Squalus acanthias*, weighing three - four kg., were maintained in a life car in seawater. Preliminary experiments were done on the common skate *Raja erinacea* and the hagfish *Myxine glutinosa*.

The basic procedure consisted of intravenous injection of 100 μCi of ^3H methoxy-inulin (New England Nuclear Corp.) and 25 μCi ^{14}C polyethylene glycol (New England Nuclear Corp.) or 25 μCi ^{14}C carboxy-inulin (New England Nuclear Corp.) and 100 μCi ^3H polyethylene glycol (New England Nuclear Corp.). Following a distribution time of 12, 48 or 168 hours the animals were sacrificed. Epaxial white muscle, liver, kidney, and gut as well as blood were removed. Each tissue was separated into four pieces. Two pieces were used for determination of tissue water by weighing before and after drying for 24 hours at 105°C. The remaining two pieces of tissue were extracted by boiling in a known volume of distilled water for several minutes and allowed to stand 24 hours under refrigeration. The tissue was then broken apart by agitation and an aliquot of the supernatant used for measurements.

In one set of experiments analysis on homogenized tissue was compared with analysis on boiled tissue. The values were identical. ^3H and ^{14}C were counted by liquid scintillation (Packard Tri-Carb). Sodium and potassium were determined by flame photometry (Instrumentation Laboratories, Inc.), and chloride was measured coulometrically (Buchler-Cotlove Co.). All tissue concentrations were expressed as mM per 1 tissue water.

The volumes of distribution in flounder tissues 24 hours after injection, of ^{14}C PEG, ^{3}H inulin, ^{3}H PEG, and ^{14}C inulin are shown in Figure 1. In muscle and gut tissue water ^{14}C labelled inulin and PEG are distributed in approximately the same volume while tritium gave higher values. In liver and kidney however ^{14}C PEG alone has the lowest volume of distribution. The volume of distribution for ^{14}C PEG seems to approximate the ECS in kidney and liver more closely than ^{3}H inulin space as can be seem from the fact that intracellular concentrations calculated on the basis of ^{14}C PEG give more realistic intracellular concentrations and lower standard error than the other compound (See Table 1).

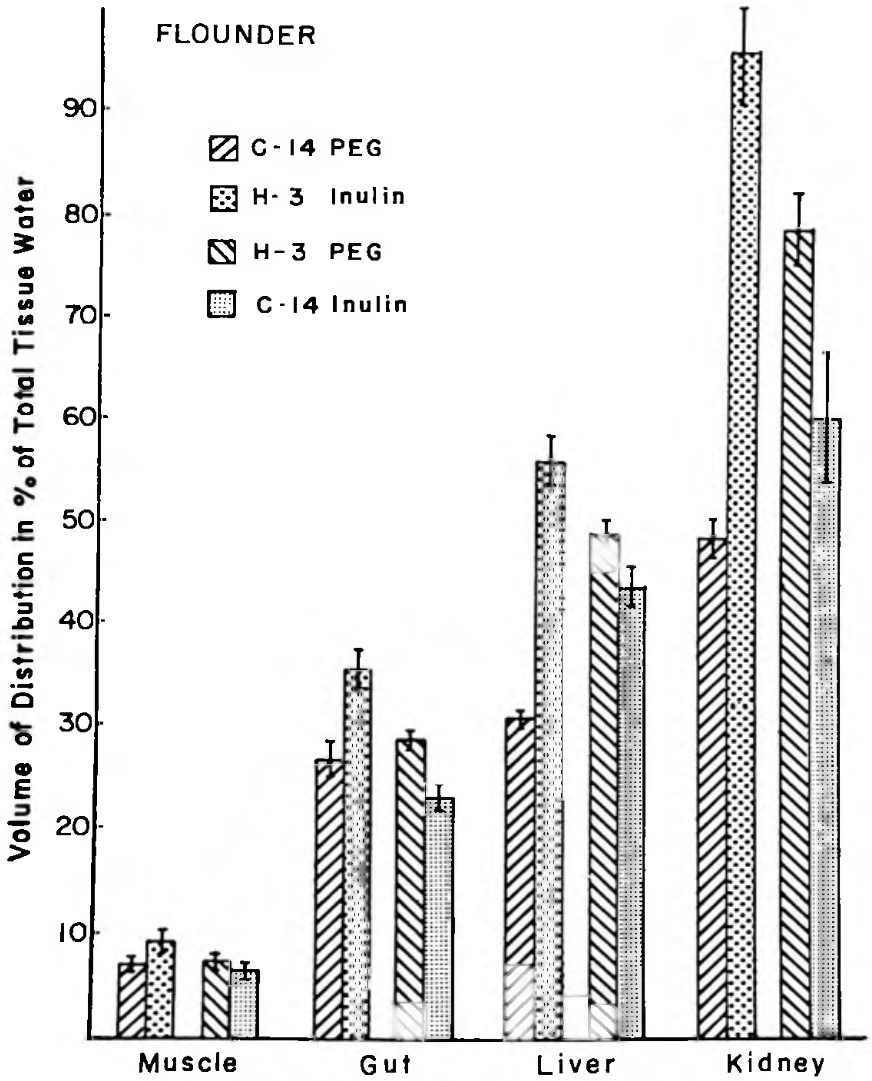

Figure 1. Flounder, *Pseudopleuronectes americanus.* Volumes of distribution of ^{14}C polyethylene glycol, ^{3}H inulin, ^{3}H polyethylene glycol and ^{14}C inulin in various tissues 24 hours after i.v. injection. Values are presented as mean of six determinations $\pm$ standard error.

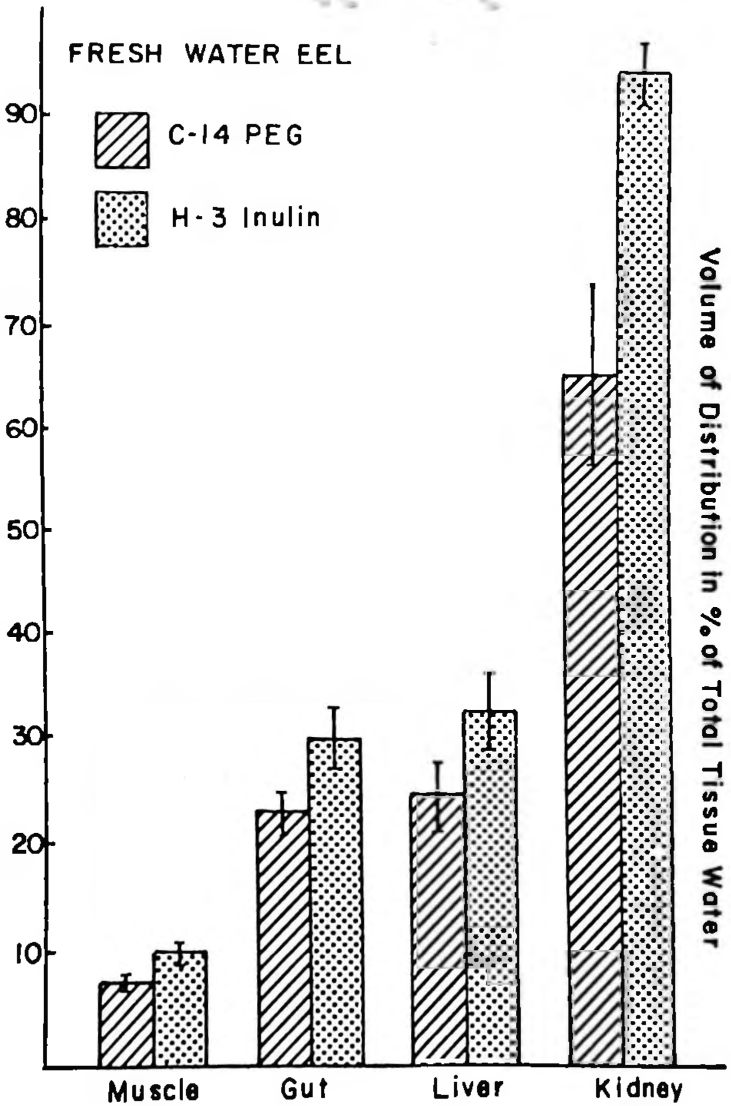

Figure 2. Eel, *Anguilla rostrata*, acclimated to freshwater. Volume of distribution of ^{14}C polyethylene glycol and ^{3}H inulin in various tissues 24 hours after i.v. injection.

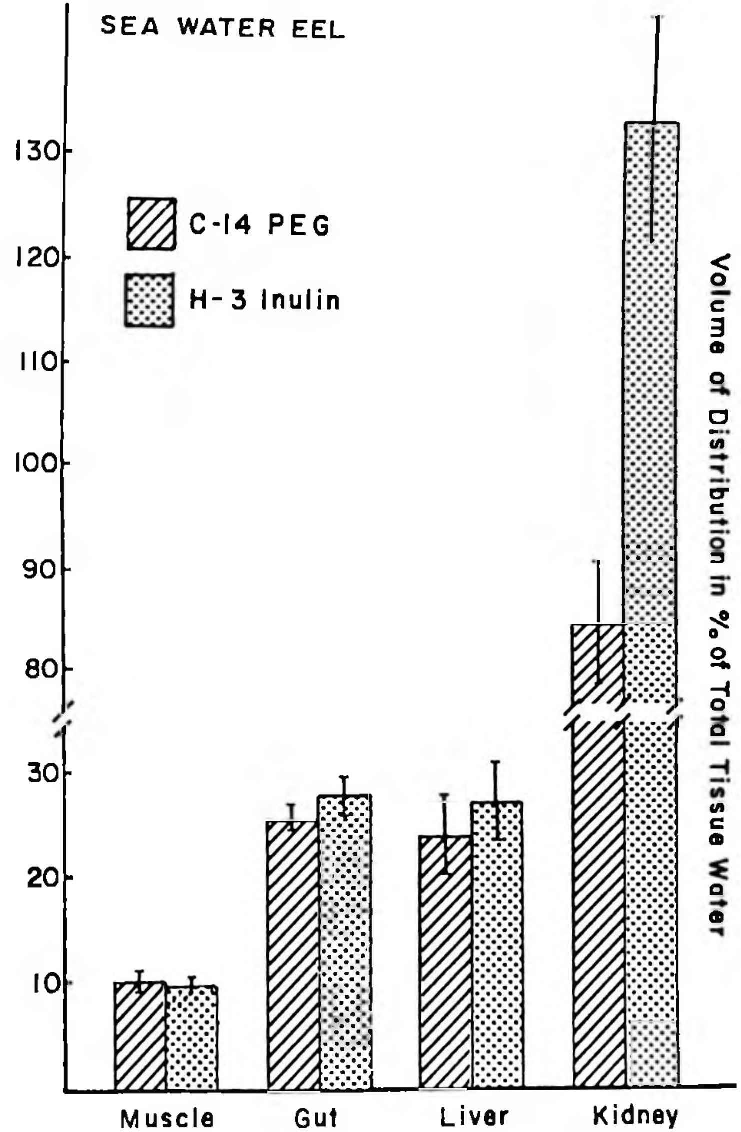

Figure 3. Eel, *Anguilla rostrata*, acclimated to seawater. Volume of distribution of ^{14}C polyethylene glycol and ^{3}H inulin in various tissues 24 hours after i.v. injection.

TABLE 1

WINTER FLOUNDER

Extracellular space (as ml per 100 ml of tissue water) and intracellular ion concentrations in liver and kidney tissue of *P. americanus*. A comparison of C-14 PEG and H-3 Inulin after *24 hours* distribution time are shown.

	Liver		Kidney	
	H-3 Inulin	C-14 PEG	H-3 Inulin	C-14 PEG
Extracellular space (ml/100 ml)	56.4±3.1* (6)	31.5±1.6 (6)	59.5±12.8 (6)	35.8±3.6 (6)
Intracellular Na (mEq/1)	-130.79±15.33 (6)	-19.58±2.00 (6)	90.10±968.16 (6)	8.11±6.05 (6)
Intracellular K (mEq/1)	289.20±24.23 (6)	181.62±7.48 (6)	29.98±1071.47 (6)	156.00±15.98 (6)
Intracellular Cl (mEq/1)	-5.17±13.02	53.35±6.09	31.19±447.66	59.36±2.57

*Mean ± standard error (n)

TABLE 2

Eel

Extracellular space (as ml per 100 ml of tissue water) and intracellular ion concentrations in epaxial muscle tissue of *Anguilla rostrata* acclimated to fresh water and salt water. A comparison of H-3 Inulin and C-14 PEG after a *168 hour* distribution time is shown.

	FW		SW	
	H-3 Inulin	C-14 PEG	H-3 Inulin	C-14 PEG
Extracellular space (ml/100 ml)	13.3 ± 1.3* (4)	13.5 ± 1.5 (4)	17.1 ± 1.1 (4)	15.2 ± 0.7 (4)
Intracellular Na (mEq/1)	6.29 ± 2.67 (4)	6.04 ± 2.69 (4)	12.01 ± 1.16 (4)	15.35 ± 1.30 (4)
Intracellular K (mEq/1)	163.65 ± 4.95 (4)	164.15 ± 5.32 (4)	176.25 ± 13.97 (4)	172.42 ± 13.22 (4)
Intracellular Cl (mEq/1)	1.37 ± 1.47 (4)	1.20 ± 1.24 (4)	7.98 ± 2.02 (4)	10.86 ± 1.68 (4)

*Mean ± standard error (n)

TABLE 3

Muscle Intracellular Concentrations
mM/1 cell water

	Extracell. Space	Na	K	Cl	Urea
Eel (S.W.)	.010 ± .009* (4)	12.41 ± 1.22 (4)	147.8 ± 0.5 (4)	9.67 ± 1.48 (4)	
Eel (F.W.)	.073 ± .009 (4)	11.94 ± .69 (4)	133.7 ± 11.5 (4)	6.80 ± 0.88 (4)	
Flounder	.068 ± .004 (6)	6.81 ± 1.15 (6)	154.1 ± 1.0 (6)	10.73 ± 1.10 (5)	
Skate	.076 (1)	7.99 (1)	172.8 (1)	10.34 (1)	359.0 (1)
Shark	.070 ± .005 (2)	28.54 ± 3.14 (2)	143.7 ± 11.4 (2)	27.62 ± 4.06 (2)	313.8 ± 0.3 (2)
Hagfish	.136 (1)	22.5 (1)	133.2 (1)	36.1 (1)	

*Mean ± standard error (n)

TABLE 4

Plasma concentrations mM/1 cell water

	Na	K	Cl	Urea
Eel (S.W.	156.9 ± 1.9* (5)	3.72 ± 0.22 (5)	135.2 ± 2.8 (5)	
Eel (F.W.)	144.6 ± 3.7 (5)	3.13 ± 0.31 (5)	101.3 ± 5.6 (5)	
Flounder	166.5 ± 2.9 (10)	3.67 ± 0.14 (10)	151.9 ± 1.4 (10)	
Skate	292.0 (1)	4.1 (1)	262.7 (1)	350.2 ± 5.0 (4)
Shark	292.7 ± 8.3 (2)	4.55 ± 0.05 (2)	278.7 ± 1.7 (2)	311.0 ± 1.0 (2)
Hagfish	431.0 (1)	11.55 (1)	421.04 (1)	

*Mean ± standard error (n)

TABLE 5

Shark, *Squalus acanthias*, Intracellular Concentrations mM/1 cell water

	Extracellular space	Na	K	Cl	Urea
Muscle	.070 ± .005* (2)	28.54 ± 3.14 (2)	143.72 ± 11.38 (2)	27.62 ± 4.06 (2)	313.79 ± .26 (2)
Liver	.027 ± .022 (4)	50.07 ± 3.25 (4)	135.54 ± 6.6 (4)	75.56 ± 5.54 (4)	325.74 ± 12.35 (4)
Gut	.323 ± .005 (4)	59.22 ± 2.60 (4)	112.02 ± 4.24 (4)	103.98 ± 2.84 (4)	357.20 ± 14.87 (4)
Spleen	.283 ± .026 (4)	35.57 ± 4.14 (4)	139.93 ± 2.99 (4)	83.94 ± 6.58 (4)	344.18 ± 3.89 (4)
Rectal gland	.242 ± .016 (2)	77.71 ± 26.76 (2)	147.98 ± 15.98 (2)	101.34 ± 2.30 (2)	352.46 ± .89 (2)
Kidney	.389 ± .016 (4)	28.98 ± 7.1 (4)	159.04 ± 6.13 (4)	28.69 ± 9.76 (4)	330.51 ± 17.25 (4)

*mean ± standard error (n)

In freshwater acclimated eel ^{14}C PEG 24 hours after injection was distributed in a smaller volume than ^{3}H inulin in all tissues (Figure 2) in seawater acclimated eels, the two volumes of distribution were more closely equivalent in muscle, gut, and liver (Figure 3), while kidney showed accumulation of both compounds.

Following 168 hours distribution time, ^{14}C PEG and ^{3}H inulin both showed exaggerated distribution values (Table 2.)

From the data given above it was concluded that tritiated labelled compounds are unreliable as measures for extracellular space since they probably are metabolized to some degree in liver and kidney. Inulin itself whether labelled with ^{14}C or ^{3}H accumulates or is metabolized in kidney and liver cells. ^{14}C labelled PEG space appears to give the most reliable measure of extracellular space but its volume of distribution should be measured within 12 to 24 hours.

Data on muscle, intracellular concentrations in various fishes are given in Table 3. The calculations are based on ECS calculated as ^{14}C PEG distribution volume. In eel, flounder, skate, and hagfish, the distribution time was 24 hours. In shark it was 12 hours. The extracellular space is given as fraction of tissue water. In view of the fact that the plasma concentrations among these fishes show such great differences (Table 4), the intracellular ion concentrations are remarkably constant.

In Table 5 the intracellular concentrations in the various organs of *Squalus acanthias* are given.

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