TABLE 4

Reversibility of effects of oil WSF and DNP on sand dollar development

Medium[a]		Developmental Effects
SLC	100% WSF	No cleavage
	50% WSF	75% formed blastulae, then died
BC	100% WSF	No cleavage
	50% WSF	75% formed blastulae, then died
DNP	10^{-3} M	Slowed development to abnormal gastrula, then died
	10^{-4} M	Slowed development to abnormal late gastrula, then died

[a]Embryos were placed in toxic medium 1 hour post-fertilization and removed to sea water 1 hour later.

EFFECT OF DILUTION ON BETA-ALANINE FLUXES IN SKELETAL MUSCLE OF *Raja erinacea*

D. Erlij, Department of Physiology, SUNY Downstate Medical Center, Brooklyn, New York

When marine elasmobranchs enter an environment more dilute than sea water, the solute concentrations in both the extracellular and intracellular fluids are markedly reduced (Forster and Goldstein, Am. J. Physiol. 230:925-931, 1976). Several amino acids are among the intracellular solutes whose concentration is most markedly reduced. This finding raises the question of whether a change in the transport mechanism for amino acids is involved in the cellular response to dilution.

In this communication I describe an initial series of experiments in which the effects of changing extracellular osmolarity on the fluxes of beta-alanine across the muscle membrane were determined. These fluxes were selected for study because it has been found in skate muscle (Boyd, Cha, Forster and Goldstein, J. Exp. Zool. 199:435-442, 1977) that this is one of the amino acids whose concentration is more drastically reduced after dilution, moreover it appears that this amino acid is not metabolized by muscle tissue (Goldstein, Personal Communication).

Bundles of muscle fibres were obtained from the pelvic fin of *Raja erinacea*. These muscles run between two tendons that can be readily dissected. To obtain pieces of tissue suitable for flux experiments bundles of fibres weighing less than 100 mg were isolated under the dissecting microscope. Care was taken to remove all the fibres that appeared damaged. The electrical excitability of the bundles was checked after dissection and at the end of the experiment.

For the flux determinations the bundles were first incubated in 4 ml of a labeled amino acid solution for one hour. Immediately after loading the muscles were soaked in a series of tubes containing 3 ml of nonradioactive solution for carefully timed intervals. The solutions were then counted to determine the radioactivity leaving the muscle during each period. At the end of the experiment the muscles were weighed and prepared for counting by dissolving them in 0.3 ml of Nuclear Chicago Solvent. By adding up in reverse order the corrected counts of the effluents to the radioactivity remaining in the bundle at the end of the experiment it was possible to determine the radioactivity present in the muscle at the midtime of each collecting period. Graphical analysis of these results (see Figure 1) showed, that the efflux process is made up of the sum of two exponentially decaying components. This analysis taken together with previous measurements of isotope washout from skeletal muscle (Harris, J. Physiol. 166:87-98, 1963) indicates that the fast component corresponds to washout of the labeled amino acid from the extracellular space while the slow component represents efflux from the intracellular compartment. According to this analysis the extrapolated intercept of the slow component corresponds to the amount of intracellular amino acids at the end of the incubation in the loading solution.

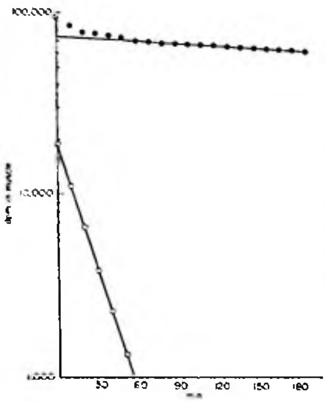

Figure 1. Washout of beta-alanine from *Raja* fin muscle. The ordinate is the total amount of radioactivity remaining in the bundle at any time. The open circles were calculated by subtracting the slow exponential from the total curve (filled circles). Only one compartment is discernible after 3 hours of washout; its intercept at zero time is taken as the initial uptake.

We compared beta-alanine fluxes among three groups of muscles:

1. Group NR/SW: normal elasmobranch Ringer was used to determine fluxes in muscles obtained from animals living in normal sea water.
2. Group DR/SW: dilute elasmobranch Ringer and muscles from animals living in normal sea water.
3. Group DR/DSW: dilute Ringer and muscles obtained from animals adapted to 1/2 diluted sea water (see Boyd et al. 1977, for composition of Ringer and of diluted sea water).

In Table 1 are shown the results of uptake determinations in the three groups of muscles; each bundle was obtained from a different fish. The uptake of beta-alanine was significantly reduced in both groups of muscles in which the determinations were made in diluted Ringer solution; the depression appeared to be more marked in the group of muscles that had not been previously adapted to the dilute environment.

TABLE 1

Effect of dilution on the uptake of beta-alanine by muscles of the pelvic fin of *Raja erinacea*

Group	(1) NR/SW	(2) DR/SW	(3) DR/DSW
Uptake µmole/g/hr	0.0284 ± 0.0024	0.0110 ± .0009	0.0223 ± 0.0018

N=6. The uptake solutions contained 0.1 m mol of beta-alanine.
Comparison of group 1 with groups 2 and 3 gave P values smaller than 0.05 in both cases.

These observations indicate that dilution of the extracellular space has a relatively rapid effect on beta-alanine uptake, since the muscles in group DR/SW had only been exposed to a dilute medium for one hour before making the uptake determination.

We also determined the effects of dilute solutions on the beta-alanine efflux from six bundles isolated from animals living in sea water. The initial efflux was always determined in normal Ringer and then followed in dilute Ringer's solution. The resting rate constant for efflux had an average value of $.00053 \pm .00004\ min^{-1}$ (n=6). The transfer of the tissue to dilute Ringer solution consistently produced an increase in efflux, however, it was always of small magnitude; between 10 and 15% of the resting efflux rate.

In conclusion, since dilution of the extracellular space can reduce to less than half the rate of beta-alanine uptake by skate muscle, it is possible that changes in amino acid transport may play an important role in the cellular adjustments to osmotic variations.

I thank Dr. L. Goldstein for his helpful suggestions.