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%.

A most interesting observation was made on the effect of salinity on the incorporation of amino acids. Embryos of late stage, incubated two days in distilled water and showing no morphological effects, were virtually incapable of incorporation of lysine into protein. The incorporation occurred and increased rapidly as the sea water content was raised, reaching a plateau at 12.5% which remained constant up to 50% sea water. Using full strength sea water, the incorporation of lysine into protein was double the plateau rate. This phenomenon deserves further study regarding both the morphological effects caused by early deprivation of sea water and the role of sea water components on the intracellular control of protein synthesis.

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1967 #9

EXCHANGEABLE OXYGEN STORES IN THE HARBOR SEAL (Phoca vitulina) DETERMINED USING O^{18} DILUTION

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The magnitude of available O_2 stores is an important determinant of survival time following loss of external O_2 supply. Presumably the ability of the seal to survive 20 minutes of diving depends on the magnitude of its oxygen stores. Previously there has been no experimental method to determine whole body exchangeable oxygen stores.

This laboratory (J. Clin. Invest. 46:1048, 1967) developed a method for the determination of exchangeable oxygen stores in man using the dilution of O^{18} in a rebreathing circuit. Total body oxygen stores (TBO_2) were calculated using the expression:

$$TBO_2 = V_i \left(\frac{F_i{}^{18}O_2 / F_i{}^{16}O_2}{F_t{}^{18}O_2 / F_t{}^{16}O_2} \right) - 1$$

where V_i represents the initial volume of oxygen in the external system determined by direct measurement, and $F_i{}^{18}O_2/F_i{}^{16}O_2$ and $F_t{}^{18}O_2/F_t{}^{16}O_2$ refer to initial and final specific activities of $^{18}O_2$ determined by mass spectrometry.

TBO_2 equals the sum of oxygen in the lung (LO_2) and non-lung oxygen (NLO_2) stores. The dilution of neon was used to measure lung volume at the time of study thus allowing calculation of LO_2, and by difference, NLO_2.

A rebreathing circuit was designed to use with the seal, and 12 measurements of oxygen stores were made in three young harbor seals, and values expressed at BTPS. Results in the seal were $TBO_2 = 48 \pm 6$ (S.D.) and $NLO_2 = 28 \pm 3$ (S.D.) ml/Kg body weight. On a weight basis, the NLO_2 of the seal was more than twice the value found in man. Multiplying estimated O_2 consumption during diving by maximal diving time suggests that death occurs when O_2 stores become entirely exhausted. The increased O_2 stores found in this animal appears to be an important adaptation subserving the function of survival during prolonged diving.