2) For 2 fish, influx values were 0.93 and 0.70 m moles/kg hr (compare Burger and Tosteson).

3) For 3 fish, efflux values were 0.13, 0.17, and 0.17 m-moles/kg hr. Thus the ratio of influx to efflux was about 5.

4) In 2 fish, the influx by skin alone was 0.8 and 1.9 m-mole/m^2 hr giving an average of 1.4 m-mole/m^2 hr. Using Boylan's figure for gill area (0.37 m^2/kg, John Boylan, personal communication) and assuming that the influx through the head is largely through the gill gives an average value for the gill influx per unit area of 2.2 m-mole/m^2 hr. This value is of the same order of magnitude as that of the skin.

In one experiment, the bag was ligated so tightly as to seriously impede circulation as determined by terminal cardiac puncture (low vol & low hematocrit). Here recoverable Na^{22} was absent until end of experiment indicating that plasma levels are a complex of the rate of penetration and later distribution by the circulating blood.

5) Ouabain did not block the uptake of Na^{22}.

6) Attempts to measure potential difference between sea water and blood (arterial and venous) failed because of A.C. interference.

While many, more precise measurements need to be taken, these data block out provisionally the magnitude of influx and efflux by the head end of the fish. The skin alone is not impermeable to Na, but its contribution is minor compared to the larger gill area. The fact that the skin alone is sodium permeable and has the same order of magnitude of permeability as does the gill seems to dispose of the possibility that in these head uptake experiments the sodium enters primarily by drinking small amounts of sea water. These data do not settle the matter as to whether uptake is passive or active. Further work is projected.

1966 #8

EFFECT OF ACTINOMYCIN D AND CYANIDE ON PROTEIN SYNTHESIS IN EMBRYOS OF Fundulus heteroclitus

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One of the central problems of embryology involves the mechanisms controlling the synthesis, regulation, and utilization of information for differentiation and morphogenesis. Presumably, the information carrier for cellular maintenance and special functions arises from genomic activity and is messenger RNA. This information is subsequently expressed by the activity of protein for which the RNA has been encoded. The investigations reported here have been concerned with the metabolic parameters which underlie the synthesis of RNA and protein during the early stages of development of the teleost embryo, Fundulus heteroclitus.

Previous studies of these investigators (Develop. Biol. 7:578-94, 1963 and Exptl. Cell Res., in press) demonstrated that: (1) Fundulus embryos develop normally to the blastula under anaerobic conditions or in cyanide; (2) the embryos are reversibly inhibited in post-blastular development while in cyanide; (3) ATP concentration is maintained at normal levels while the pre-blastular embryos are in cyanide, but is reduced reversibly at later stages; (4) the rate of pre-blastular glycolysis is increased by cyanide to maintain a normal rate of ATP synthesis while at

later stages the glycolytic rate in cyanide cannot maintain the normal ATP synthesis rate; (5) cyanide completely and reversibly inhibits RNA synthesis at all stages of Fundulus development; (6) Actinomycin D, while not affecting cleavage and blastulation, inhibits further development if added to the embryo medium within the first hour post-fertilization; (7) the effect of Actinomycin D can be postponed by incubating the embryos in cyanide immediately after fertilization. Thus it became clear that under conditions where oxygen consumption is inhibited, RNA synthesis is also inhibited even though normal levels of ATP are maintained. Furthermore, the inability to synthesize RNA had no effect on normal cleavage and blastulation, but did prevent further development. Therefore, the RNA required for cleavage and blastulation must exist in the unfertilized egg and is long-lived (approximately 40 hours).

It was of interest to evaluate the effect of these inhibitors on protein synthesis in the cleavage stage embryos. Presumably, cleavage and blastulation require some level of protein synthesis and in light of previous data this must go on even though RNA synthesis is inhibited. Thus attention was focused this summer on the effect of anaerobiosis, cyanide, and Actinomycin D on the incorporation of amino acids into embryo protein.

The extent of amino acid incorporated into protein was measured as the amount of radioactivity in protein isolated from embryos which had been incubated in the presence of labeled amino acids. Embryos were preincubated in the inhibitor (20 μg/ml Actinomycin D or 2×10^{-3} M NaCN) for 30 minutes after which the labeled amino acid was added and incubated for 2 hours. The trichloroacetic acid-insoluble fraction was washed free of soluble labeled material, heated, and finally extracted with organic solvents. The resultant protein precipitate was dissolved in 0.1 M NaOH from which aliquots were removed for counting in a gas-flow planchet counter.

Incorporation into protein and the effect of inhibitors was found to be dependent on the amino acid used and the developmental stage. Lysine incorporation was greatly stimulated by both cyanide and Actinomycin D during early cleavage stages. At later stages lysine incorporation was completely inhibited by cyanide and partially inhibited by Actinomycin D. Phenylalanine incorporation, always at a very low level, was partially inhibited by cyanide at early stages but greatly stimulated by cyanide after gastrulation. Actinomycin D did not affect phenylalanine incorporation until gastrulation, after which it caused some stimulation. Leucine incorporation was partially inhibited by cyanide until gastrulation after which it was completely inhibited. Actinomycin D caused a mild inhibition of leucine incorporation. An amino acid mixture responded to the inhibitors in a manner similar to leucine.

It thus became apparent that the response of amino acid incorporation to the inhibitors was dependent upon the amino acid precursor and the stage of development. This would indicate that different proteins are being synthesized at different stages and that the sensitivity of these syntheses to blockage of RNA synthesis and anaerobic metabolism is stage dependent. Post-blastular protein synthesis was completely blocked by cyanide which is in accord with the morphological data which show arrested development. However, phenylalanine incorporation is an exception, actually being stimulated by cyanide in the post-blastula. This may be due to the onset of pigment synthesis which requires phenylalanine. It is not known whether pigment synthesis is sensitive to cyanide. The data support the possibility that some protein synthesis occurs during cleavage stages even though aerobic metabolism and RNA synthesis are completely arrested. It is particularly interesting to note that lysine incorporation is even stimulated by the inhibitors during cleavage stages. This might suggest that basic proteins required for chromosome synthesis may

be synthesized during early embryogenesis in the absence of both new messenger RNA synthesis and aerobic metabolism. (These investigations were supported by Grants HD-00519 and DE-02047 from the U.S.P.H.S.)

1966 #9

THE DISASSOCIATION OF BRADYCARDIA AND ARTERIAL CONSTRICTION IN THE DIVING SEAL, Phoca vitulina

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The ability of the seal to remain submerged in water without access to external oxygen source for periods of 20 minutes is accomplished by circulatory adaptive changes that are referred to as the "dive response." These adaptive changes are bradycardia with pronounced decrease in cardiac output (Amer. J. Physiol. 210:176-80, 1966), and arterial constriction that prevents blood perfusion to peripheral tissues (Amer. J. Physiol. 135:557-66, 1942).

In order to evaluate the relative roles of these two circulatory responses in the adaptation to diving it is important to disassociate them during diving. Attempts in the past to individually block one or the other response using a pharmacologic approach have not been successful. Atropine will prevent the dive response, but does not allow individual evaluation of the bradycardia or of the arterial constriction in response to diving.

In this study, the technique of electrical cardiac pacing was used to regulate the heart rate of the seal while out of water and during diving to disassociate arterial constriction from bradycardia. Four harbor seals were trained to dive under laboratory conditions using a teeter board to control the time of submersion (J. Cell. & Comp. Physiol. 58:261-66, 1961). Using procaine for local anesthesia, an intracardiac pacing electrode (Elecath pacing stylet, #550, size 0.034", length 39 cm) was inserted through the chest wall, via a thin wall 18 gauge needle, and positioned in a cardiac chamber. The intracardiac pacing electrode was connected using insulated leads to a battery operated pacemaker (Medtronics, model 5800) with rate and amperage adjustable from 50-180/minute and 1.1 to 22 milliamps respectively. A polyethylene catheter (PE 90) was inserted into a femoral artery and advanced into the aorta. Arterial pressure was monitored using a Statham strain gauge and a polygraph recorder.

Three types of studies were performed. Control dives without cardiac pacing were conducted to ascertain that bradycardia occurred normally. In the second type of study the seal was dived and pacing instituted after the onset of bradycardia to demonstrate that bradycardia could be stopped during diving. In the third type of study, pacing was instituted prior to the dive and bradycardia was prevented for dives of over six minutes in duration. In one seal, arteriograms were performed using a technique previously described (Science 152:540-43, 1966). The arteriograms were performed during normal diving and during diving when bradycardia was prevented by cardiac pacing.

It was found that the heart rate could readily be controlled by the pacemaker during diving. The arteriograms revealed striking arterial constriction during diving with and without bradycardia. The ability of the seal to dive for six minutes without occurrence of bradycardia demonstrated that the onset of the arterial constriction is not dependent upon bradycardia and that ar-