Table 1

RELATIVE COUNTS OF TOTAL $^{14}CO_2$ IN THE DOGFISH

	Plasma		CSF	
	5 min	12 min	5 min	12 min
Control	100	37	20	40
Treated*	100	45	6	11
	100	56	18	29

*30 mg/kg acetazolamide i.v. 30 minutes before injection of $NaH^{14}CO_3$.

are obtained; at this time the value is 8. This number can be entered as the substrate (S) in the rate equation for the uncatalyzed reaction

$$V_{unc.} = (S)\, k_1$$

where k_1 is the hydration rate constant at 14° C, 0.7 min^{-1}. Thus the uncatalyzed rate would provide the accumulation of 5.6 units per minute. We find from the table that the control rate (from 5 to 12 minutes) was 3 units per minute; treated fish were less but the same order of magnitude. Thus there appeared no catalytic component; this would, if present, make the HCO_3^- accumulation instantaneous, as in the eye (Kinsey and Reddy, vide supra). The fact that the physiological rates are comparable to the noncatalytic suggests that we are seeing an inevitable chemical process—akin to CO_2 being generated into a buffer—and not one of importance in physiological regulation. The non-participation of carbonic anhydrase in this process is borne out by the fact that the rates in the inhibited fish show no great (although there is some) difference from the controls. The two treated fish show variability and clearly more experiments are needed. Tentatively, the data suggest that carbonic anhydrase has a different role in CSF chemistry—perhaps associated with Cl^- or H^+ secretion, as in the stomach and salt gland (Phys. Rev. 47:595, 1967).

In conclusion, it appears that the rates of $H^{14}CO_3^-$ appearance (and those of other isotopes) in CSF can readily be studied in S. acanthias, over a brief period of time. Data thus far point away from a primary process involving plasma $CO_2 \rightarrow$ CSF HCO_3^-, since carbonic anhydrase is not involved in the reaction.

1968 #8

CONDITIONS OF INHIBITION OF MORPHOGENESIS AND MACROMOLECULAR SYNTHESES IN Fundulus heteroclitus EMBRYOS

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The determination of conditions under which embryogenesis is altered or inhibited has long been useful in the search to understand the normal process. Preliminary studies on certain in-

hibitors and culture modifications have indicated the necessity to further correlate effects on macromolecular syntheses and morphogenesis (Bull. MDIBL 7:14-15, 1967).

These studies were conducted on embryos of the teleost Fundulus heteroclitus which were fertilized and cultured as described previously (Exptl. Cell Res. 44:471-88, 1966). Protein synthesis was measured as the extent of incorporation of ^{14}C-labeled amino acids into hot trichloroacetic acid-insoluble material during incubation of the embryos. RNA synthesis was measured as the extent of incorporation of uracil-2-^{14}C into hot trichloroacetic acid-soluble material.

Pactamycin was investigated for its effect on protein synthesis and on morphogenesis. Optimal inhibition of amino acid incorporation, which was approximately 85%, occurred at a level of 20 μg/ml at all stages of development. This inhibition proved to be reversible, full recovery being observed about four hours following removal of the inhibitor. The effects of this inhibitor on morphogenesis varied depending on duration of pulse and time of pulse initiation. At least two cleavage generations occurred in pactamycin and if the incubation period was less than nine hours the embryo would ultimately recover and develop normally. Exceptions to cleavage ability and reversibility occurred during the first four minutes post-fertilization. The results on early development were inconsistent and further studies are required but it does seem clear that temporal control over protein synthesis is especially critical during the first few minutes following fertilization.

Since the inhibitory action of pactamycin proved to be reversible, attention was given to the ability of embryos to resume RNA synthesis following removal from Actinomycin D. It was found that embryos incubated in Actinomycin D for two hours and removed to normal media for one day had recovered 95% of their ability to incorporate uracil into NRA. This reversibility of Actinomycin D effect was demonstrated in late stage embryos where it had already been shown that the two hour pulse of the inhibitor had no effect on morphogenesis. It will be of interest to test the reversibility of Actinomycin D action at cleavage stages where morphogenesis is affected.

Previous studies had shown that cyanide allows cleavage of Fundulus embryos while reversibly inhibiting all development beyond the blastula. Calculations based upon metabolic studies demonstrated that during the cleavage stages ATP synthesis could be maintained at normal levels due to a compensatory elevation of the rate of glycolysis (Exptl. Cell Res. 44:453-70, 1966). It was revealed through further studies using cyanide that this inhibitor completely prevented amino acid incorporation into protein at all stages (Exptl. Cell Res. 44:489-97, 1966). Therefore it was of interest to determine whether recovery of protein synthesis would occur during cleavage stages upon long incubation in cyanide, the time period when anaerobic ATP synthesis fully compensates for the loss caused by inhibiting aerobic metabolism. Thus protein synthesis activity was measured in embryos kept in cyanide for as long as four days. In all cases, amino acid incorporation was completely inhibited and there was no evidence for recovery while remaining in cyanide. Thus it appears that the inhibition of amino acid incorporation into protein by cyanide in Fundulus embryos is not caused simply by repressed synthesis of ATP.

The effect of varying the degree of salinity of the incubation medium on amino acid incorporation into embryo protein was further investigated. Sea water concentrations of 0, 6.25, 12.5, 25, 50, 75, and 100% were used. Embryos were preincubated in these various media for two hours after which a mixture of fifteen ^{14}C-labeled amino acids was added and allowed to incorporate for two hours. Various stages of embryos gave similar results. A minimal incorporation

occurred at the lower salinity levels. A fourfold increase occurred in 25% sea water and at 50 and 75% levels a 100-fold increase in incorporation was seen. The embryos in 100% sea water showed another sharp increase, their incorporation being 160-fold greater than the low salinity embryos. These findings were in marked contrast to the results of experiments on the effect of salinity on development. Regardless of the salinity of the incubation medium, embryos developed normally to hatching stages.

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1968 #9

CEREBROSPINAL FLUID (CSF), AN EXCRETORY SYSTEM FOR BRAIN

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This report confirms and extends preliminary experiments suggesting that substances may be removed from brain by net diffusion into CSF (sink hypothesis of CSF function).

When plasma inulin concentration is maintained constant in Squalus acanthias, steady state inulin concentration in brain extracellular fluid (ECF) is greater than in CSF (Cserr et al., this Bulletin 7:16-18, 1967). Thus CSF may serve as a sink for inulin in brain. In order to test this possibility, we have looked for inulin diffusion gradients within brain ECF.

Plasma ^{14}C-inulin concentration was maintained constant for twenty hours by repeated intravascular injections. Fish were then sacrificed and the medulla oblongata was removed rapidly and cut into three sections (rostral, middle and caudal). Inulin concentration gradients within each of the medullary sections were determined by cutting tissue (parallel to the ventricular surface) into four slices (1/2 mm thick) and analyzing for radioactivity. In order to evaluate the possibility that variations in either vascular volume or ECF volume might contribute to inulin concentration gradients within brain tissue, vascular volume was determined using ^{14}C-dextran (30 minutes after intravascular injection); and ECF volume was determined using methods described by Rall, Oppelt and Patlack (Life Sciences, 2:43-48, 1962). Results are summarized in Table 1.

Table 1

STEADY STATE CONCENTRATION GRADIENTS IN BRAIN EXTRACELLULAR FLUID (ECF)

mm from CSF	ECF Inulin concentration (plasma concentration = 100)		
	Rostral medulla	Middle medulla	Caudal medulla
0.0 - 0.5	18	24	26
0.5 - 1.0	20	26	30
1.0 - 1.5	32	31	36
1.5 - 2.0	46	40	47