

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

Demonstration that inulin concentration of brain ECF increases progressively with distance from CSF supports the sink hypothesis of CSF function. We are presently investigating the possibility that polar end products of brain metabolism may be removed from brain via net diffusion into CSF.

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1968 #10

LINEAR VELOCITY AND FLOW RATE OF TUBULAR FLUID IN SURFACE NEPHRONS OF
Squalus acanthias IN SITU.

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Dogfish weighing 1-3 kg were injected with nembutal (15 mg/kg BW) and placed ventral side up on a V board. Their gills were perfused with running sea water. Through an 18G needle placed in the dorsal aorta a polyethylene catheter (PE10) was passed retrograde to a point approximating the cephalic end of the kidneys. The right kidney was exposed through a midline abdominal incision and gentle retraction of the viscera; its surface was visualized by incident light microscopy. In fish of about 1 kg BW the renal capsule is transparent and surface tubules can be seen through it. In larger fish the capsule is often dense and a portion must be removed to allow for a satisfactory study. Since in these larger fish the capsule is also closely adherent to the kidney surface, we encountered considerable bleeding during this surgical preparation.

A bolus of Lissamine green dye (0.5 ml of 5% aqueous solution) was rapidly injected into the catheter and its passage observed.

Deep vessels, fanning out radially and segmentally from the aortic region become colored 5-10 seconds after the injection. Filtered dye appears in loops of surface nephrons 8-15 seconds later. In measured lengths of surface loops the linear velocity ($\mu \text{ sec}^{-1}$) of the dye front was recorded. The inner diameter of these loops was taken as the width of the column of dye. Tubular fluid flow rates ($\text{ml min}^{-1} \times 10^{-6}$) were then calculated from linear velocity and cross-sectional area. Representative data from 12 measurements on 5 fish are set out in Table 1.

Linear velocity of tubular fluid ranged from 90 to 280 $\mu \text{ sec}^{-1}$, tubular diameter from 33 to 50 μ and flow rate from 9 to 33 with a mean of $20 \times 10^{-6} \text{ ml min}^{-1}$.

In a second population of surface loops, more numerous than the first and lateral to it, dye appears 100 to 300 seconds after the injection.

Ghouse and Brennan (this Bulletin, abstract #15) estimate about 8,800 glomeruli in a dogfish of average size. In these authors' micro-dissection study, 26 completely dissected nephrons were found to have a mean length of 31.3 mm. It is interesting to relate our functional data with these anatomical descriptions, now available for the first time. The appearance of dye in surface loops about 8-15 seconds after it reaches the kidney locates these loops in the first 1-3 mm of the nephron and means that a flow rate measured here would approximate single nephron filtration rate. In free-swimming dogfish, 2.6 - 4.4 kg BW, the clearance of inulin was found to average 12 ml hr^{-1} (Bull. MDIBL 6:2, 1966). At 8,800 glomeruli per fish this yields a mean GFR per nephron of $22.6 \times 10^{-6} \text{ ml min}^{-1}$ compared to our mean value of $20.6 \times 10^{-6} \text{ ml min}^{-1}$ cal-