

Additional materials prepared for subsequent electron microscopic examination include spiral valve, gall bladder and spleen of Squalus and the lower intestine of Fundulus.

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1967 #12

#### BULK FLOW BETWEEN THE CEREBELLUM AND THE CEREBELLAR VENTRICLE FLUID IN Squalus acanthias

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The brain ventricular system of Squalus acanthias consists of two lateral ventricles in the olfactory lobes, a third ventricle in the midbrain, a fourth ventricle in the medulla, a cerebellar ventricle, and an optic lobe ventricle. All ventricles are communicating, thus allowing a free flow of ventricular fluid between them. The lateral, third, and fourth ventricles contain choroid plexus tissue; the cerebellar and optic lobe ventricles are devoid of such tissue. If the cerebellar ventricle can be isolated from the ventricular fluid circulation by occluding its connection to the ventricular system, it would be possible to study the transport of material between the ventricular fluid of the cerebellum, the cerebellum, and the blood uncomplicated by the presence of the choroid plexus.

Several methods were tried to obstruct the flow of ventricular fluid into the cerebellar ventricle. All methods were checked for effectiveness by injecting small amounts of fluorescent dye into the lateral and/or fourth ventricles and examining the cerebellar ventricular fluid for fluorescence. The best blockage was obtained by injecting latex into the aqueduct between the third and fourth ventricles via an opening in the optic lobes.

By placing inflow and outflow cannulae into the cerebellar ventricle after blocking the opening with latex, a perfusion system was set up. Using a  $C^{14}$ -inulin dilution technique in this system, it is possible to detect and estimate bulk flow of fluid between the cerebellum and its ventricle. The results from one experiment (six determinations) indicated little or no volume flow from the cerebellum into the cerebellar ventricle ( $0.04 \mu\text{l}/\text{min.} \pm 0.05 = \text{mean flow rate} \pm \text{standard error of the mean}$ ). Oppelt, Patlak, Zubrod, and Rall (Comp. Biochem. Physiol. 12:171-77, 1964) measured a total ventricular fluid production rate for Squalus of  $4 \mu\text{l}/\text{min}$ . It appears that the principal site (or sites) of ventricular fluid production is (or are) not located in the cerebellum.

#### NITROGEN METABOLISM IN FISH: BLUTAMATE DEAMINATION AND AMINO ACID TRANSDEAMINATION BY EEL (Anguilla rostrata) LIVER MITOCHONDRIA

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Crude homogenates of eel liver were previously demonstrated to deaminate glutamate and transdeaminate alanine (McBean, Neppel, and Goldstein, Comp. Biochem. Physiol. 18:909, 1966). We extended these observations to isolated liver mitochondria in this study. Mitochondria were

prepared by differential centrifugation of sucrose homogenates. The incubation medium was similar to that used for rat liver mitochondria (Hird and Marginson, Arch. Biochem. Biophys. 115:247, 1966). Flasks were shaken in air at 25°C and ammonia production was measured by a microdiffusion-colorimetric technique. The rate of glutamate (10mM) deamination by eel liver mitochondria was approximately 1  $\mu$ mole/g liver per 30 min at 25°C as compared to a rate of approximately 2  $\mu$ moles/g liver per 30 min for rat liver mitochondria at 38°C. In contrast to previous findings with rat liver mitochondria (Hird and Marginson), an ADP generating system was not essential for glutamate deamination by eel liver mitochondria. Deamination of alanine and aspartate by eel liver mitochondria was increased several fold by the addition of  $\alpha$ -ketoglutarate, indicating that the pathway of ammonia production from these amino acids is transamination with  $\alpha$ -ketoglutarate to produce glutamate and subsequent deamination of the latter amino acid. No transdeamination of glycine, phenylalanine, lysine, or leucine was observed.

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#### NITROGEN METABOLISM IN FISH: EFFECT OF SALINITY ON UREA BIOSYNTHESIS IN THE SKATE (Raja erinacea)

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The early studies of Smith (Am. J. Physiol. 98:279, 1931) showed that blood urea concentration was significantly lower in fresh water elasmobranchs than in marine forms, a condition which aids in osmotic regulation. An increase in urine flow is known to contribute to the reduction in blood urea, but the role of urea biosynthesis in this phenomenon is unknown. We, therefore, investigated the effects of salinity on urea biosynthesis in the skate, Raja erinacea. Four skates weighing 380-900 g were maintained in a 12 foot swimming pool in running water at 12°-15°C. After one week in full strength sea water the rates of urea and ammonia excretion (production) were  $213 \pm 44$  (S.E.) and  $123 \pm 47$   $\mu$ moles/kg x hr. Diluted sea water was then added to reduce the salinity to 78% over a period of three days. The skates were maintained at this salinity for an additional three days. The rates of urea and ammonia excretion and blood urea concentration were determined on the last day. Plasma urea concentration was  $332 \pm 12$   $\mu$ moles/ml as compared to a value of  $395 \pm 11$   $\mu$ moles/ml in a separate group of skates maintained in sea water ( $p < .02$ ). The rate of urea excretion in skates in dilute sea water was  $457 \pm 55$   $\mu$ moles/kg x hr which was significantly higher than the rate observed in sea water ( $p < .05$ ) by "paired-data" analysis. The rate of ammonia excretion ( $181 \pm 37$   $\mu$ moles/kg x hr) was similar to that observed in sea water ( $p < .1$ ).

If it is assumed that urea excretion equates to urea production under these conditions, the rise in urea production in skates maintained in diluted sea water is surprising since this would tend to oppose the mechanisms operating to lower blood urea concentration. It is possible that the predominant stimulus for urea biosynthesis is not osmotic pressure but rather blood urea concentration.

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