

3) The existence of an electron transport chain may be inferred from the inhibition of  $O_2$  consumption by cyanide (inhibits cytochrome  $a_3$ ) and antimycin (inhibits cytochrome b), but this electron transport chain is not organized in the usual structural form since mitochondria are not present.

4) The cellular sites and energy requiring processes for  $O_2$  utilization are not known. This work was supported by USPHS Grants 10061-01 and 05059-07.

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#### MICROPUNCTURE STUDY OF UREA MOVEMENTS ACROSS THE RENAL TUBULES OF Squalus acanthias

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Urea is actively resorbed in the renal tubules of elasmobranchs as shown by the fact that the urine urea concentration is considerably lower than that of the blood. In the dogfish the urea U/P is 0.1 to 0.2 while the inulin U/P is 3 to 8, thus 90 to 99% of the filtered urea may be resorbed.

The purpose of the investigation was to localize the tubular site of active transport and to determine the rate of transport, and the permeability to urea. Theoretically, this can be done in two steps:

1) A test solution (containing a non resorbable solute, such as, manitol) is introduced into the tubule between oil droplets and left until a steady state has been reached where the unidirectional fluxes in and out of the tubules will be equal. If active transport out of the tubule is present, concentration in the tubule will be lower than in the blood. I.e., the active and passive out-flux will equal the passive influx.

2) The unidirectional flux is measured with labeled urea at the steady state concentration.

The dorsal surface of the right kidney was exposed in the anesthetized dogfish. Micropuncture free flow samples were collected from renal capillaries and proximal tubules. Free flow samples were also collected from the ureter by catheterizing them with polyethylene tubing and from the bladder. Urea was measured by the micromethod of Marsh on nanoliter samples. The concentrations found were approximately: renal plasma, 350 mM/l; proximal tubule, 350-360 mM/l; ureter, 40 to 100 mM/l; bladder, 40-100 mM/l. It was not possible to obtain free flow samples from the distal tubules because of difficulties in visualizing them on the kidney surface. Only when the tubules were filled with the colored test solution could we see them.

To determine the stopflow concentrations a solution of 450 mM/l mannitol, 300 mM/l urea and 50 mM/l NaCl was introduced into the various parts of the tubule. Stopflow fluid/plasma ratios were: ureter, 1.05; small collecting duct, 0.92 to 1.01; distal tubule, 0.92; proximal tubule, 1.00-1.10.

The free flow collections indicate that the urea resorption takes place between the proximal tubules and the collecting ducts, i.e., in the distal tubules. The stopflow determinations have failed to show a clear difference between distal tubular fluid and blood. The experiments are not finished and it is quite possible that we have failed to identify the distal tubules properly.