The acidification rates observed in these *in situ* micropuncture experiments agree reasonably with those found in the free swimming fish. We have shown the rate to be 40 μmoles/L cell volume per minute *in situ*. The excretion of titratable acid in the related elasmobranch fish *S. acanthias* is about 0.2 μmoles per minute per kg body weight (Hodler et al., vide supra). Using this figure (which is probably high for *R. erinacea* because of its lower GFR and metabolism than *S. acanthias*) and a kidney cell volume of 1.5 ml for a skate weighing about 1 kg yields 133 μmoles/L cell volume.

In these species lacking carbonic anhydrase it therefore appears that essentially all HCO_3^- is reabsorbed as such and H^+ secreted by tubule cells inevitably appears in the urine as titratable acid.

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1972 #10

UREA REABSORPTION BY THE SKATE NEPHRON: MICROPUNCTURE OF COLLECTING DUCTS IN *Raja erinacea.*

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Before entering a collecting duct the final thin-walled segment of the skate nephron passes in intimate apposition to coiled loops of more proximal segments (Bull. MDIBL 10:5, 1970). A similar finding in Squalus had suggested that this disposition of early and late segments might play a role in the unique handling of urea by the elasmobranch kidney (Bull. MDIBL 9:60, 1969). In the present study the urea concentration of initial collecting duct fluid was compared to that of plasma and simultaneously formed urine. Results indicate that tubular fluid entering the collecting duct has already reached its final concentration with respect to urea.

Data are presented from 13 male and female skates weighing 0.74 -1.2 Kg and examined not more than two days following capture in Frenchman Bay. Fish were anaesthetized with nembutal (0.2 mg Kg^{-1}) and curare (0.3 mg Kg^{-1}) and prepared for micropuncture as previously described (Bull. MDIBL 10:5, 1970 and Ibid. 11:91, 1971). Collecting ducts are visible and accessible at the dorsal surface of the exposed kidney. Urea determination in tubular and collecting duct fluid was by the method of Marsh (J. Clin. Path. 14:418, 1961), in plasma and final urine by the phenol-alkaline hypochlorite method. The puncture site in each case was identified by the injection of a droplet of Lissamine green or microfil after sampling was complete.

Plasma urea concentration ranged from 300-423 mML^{-1} (mean and SD, 370 ± 40); urine to plasma (U/P) urea ranged from 0.14 to 0.39. We obtained 15 samples from first order (initial) collecting ducts. Fluid: plasma ratios for these varied from 0.10 to 0.42, not significantly different from final urine.

Stolte, et al, (Bull. MDIBL 11:91, 1971) found the collecting ducts to be the principal site of urinary dilution in the skate kidney. It appears therefore from the present study that urea reabsorption and the final adjustments in ionic composition occur at different sites along the nephron of the skate. Urea is reabsorbed and has reached its final concentration before the point of entry into the collecting ducts. Sodium and perhaps other ions as well are reabsorbed in the collecting ducts, leaving a slightly hyposmotic final urine.

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1972 #11

CYTOLOGICAL EFFECTS OF MODIFIED FLUID TRANSPORT IN THE INTESTINE OF *Squalus*.

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The isolated perfused spiral valve of the dogfish provides a favorable preparation for electron microscopic study of changes in structure of the absorbing cells in relation to fluid transport conditions. Active fluid transport and intact ultrastructure can be maintained for two to four hours at controlled temperatures between 10° and 18°C with arterial and luminal perfusion under known osmotic and hydrostatic pressures and in the presence of suitable inhibitors. Complications in interpretation of the configuration of the lateral intercellular channels due to effects of adjacent smooth muscle which have been noted for other preparations are avoided in *Squalus* by virtue of the structure of the spiral fold. As shown previously (Doyle, Comp. Biochem. Physiol. 42A, 65, 1972) the effects on the sparse muscularis muscosa are confined to the basal few microns of these tall (100μ X 5μ) absorptive cells and are related to the direction of the muscle fibers. Under conditions of enhanced fluid uptake the rate of fluid transport can be correlated with the length of the intercellular channel from the base towards the apex whereas the degree of distension of the channel may be diminished by cell swelling (in hypotonic media) or expanded by cell shrinkage (in hypertonic media) or transiently affected by a peristaltic wave. When transport is blocked by $10-^4$ ouabain the cells swell and intercellular channels are obliterated. Transport blocked by hypertonic sucrose resulted in some cell shrinkage and expanded intercellular channels in basal regions between cells.

Of more immediate cytological significance is the demonstration in these tall columnar cells of the segregation of the transport processes to the apical portion of the cell internally and the relegation of the product to external spaces in the more basal region. The normal polarization of intracellular organelles shows a subnuclear zone essentially unaffected by varied rates of transport or moderate degrees of cell shrinkage or swelling. Apically, however, the density of matrix cytoplasm directly reflects swelling or shrinkage and the partition of fluid between the matrix and the endoplasmic reticulum compartments is sensitive to transport conditions. The Golgi zones are conspicuous at the boundary between the sensitive apical zone and the more stable basal region. It is at this level that extrusion of fluid from the cell is most active. In several of our preparations there was evidence of fat transport via the endoplasmic reticulum and Golgi to the lateral intercellular channels and the appearance of extracellular chylomicrons. These observations lend some support to the theoretical conclusions of Diamond (Fed. Proc. 30, 6, 1971) that the membrane density of sodium pump sites should be greatest at the blind (apical) end of the intercellular channel.