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.

Perfusion with 10^{-4} M amiloride, and incubation of excised pieces of mucosa with this drug causes derangement of the normal internal polarization of the cell. The granular endoplasmic reticulum hypertrophies and extends, atypically, into the apical cytoplasm as arrays of flattened cisternae. Transport is reduced but not totally blocked in one-half hour. Unlike the effects of ouabain, there was no evidence of cell swelling, the cytoplasmic matrix is normally dense and the interdigitations of lateral cell membranes are complex. Lateral intercellular spaces with cell projections occur, atypically, well into the apical regions but at the nuclear level are replaced by closely apposed membranes. In the perinuclear region there are multiple Golgi areas with abnormally increased numbers of flattened lamellae some of which are contorted to multilayered myelin whorls.

When the normal outflux mechanisms are deranged by treatment with ouabain or amiloride the endoplasmic reticulum gives rise to large intracellular vacuoles (or vacuoid spaces lacking membranes) which may fuse with lateral cell membranes resulting in bulk discharge of the fluid.

Little is known of the processes of transport between the cytoplasmic matrix and the interior of the Golgi-endoplasmic reticulum compartment. In normal preparations with active fluid transport our observations are consistent with an assumption of osmotic balance between these intracellular compartments. The observed morphological proportions and arrangements of these compartments are profoundly affected by both ouabain and amiloride but in distinctly different ways. The effects of amiloride warrant further investigation.

1972 #12

CYTOLOGY OF CHLORIDE CELLS IN GILLS OF THE EEL, *Anguilla;* THE STICKLEBACK, *Pungitius;* AND THE ROCK-EEL, *Pholis.*

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The structure of the chloride cells in the gills of the American eel, *Anguilla rostrata;* the nine-spined stickleback, *Pungitius pungitius;* and the rock-eel, *Pholis gunnellus,* was examined in the electron microscope in relation to conditions of osmotic adaptation, cortisol treatment, and acute poisoning with thiocyanate.

In *Anguilla*, as described by Doyle and Epstein (Cytobiologie 6, 58, 1972), the correlation shown between development of Na-K-ATPase and development of chloride cells left open the question of a possible effect of the position of the cells in the gill epithelium on the lag in ability to regulate serum electrolytes upon transfer to sea water. Subsequent experiments with freshwater adapted eels, treated for an additional ten days with cortisol and transferred to seawater for 24 and 72 hours respectively, showed that a sufficiently large number of mature chloride cells were exposed at the surface of the epithelium to rule out the time required for a shift in position of chloride cells to the surface as a cause of the delay in physiological regulation of serum electrolytes. There were however some fine structural differences between the 24 and 72 hour specimens. The 24-hour specimens showed a more highly reticulated tubular system than the 72-hour ones, suggesting that some intracellular reorganization process may be involved which requires two or three days to be accomplished.