Effect of bumetanide on solute uptake by plasma membrane vesicles isolated from rectal gland of Squalus acanthias

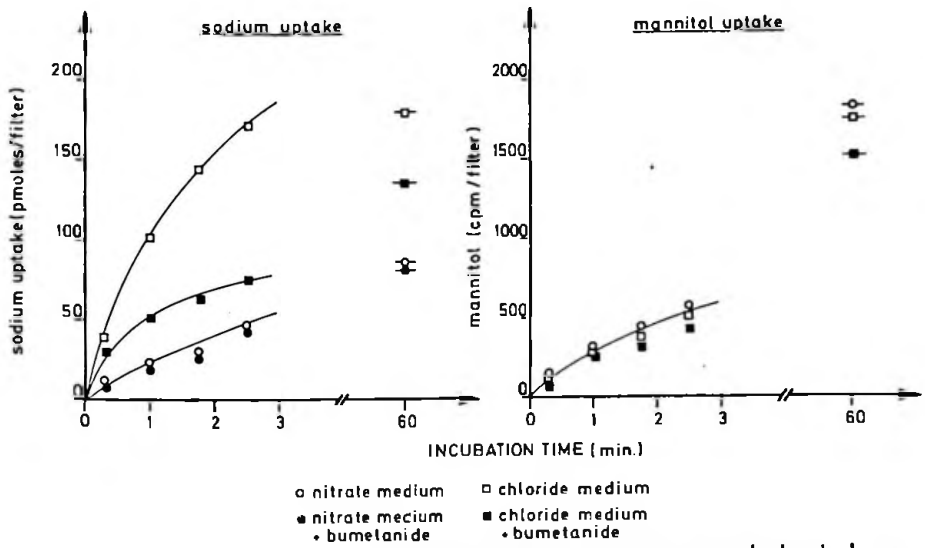

Figure 2. Effect of diuretics on the chloride dependent sodium uptake into rectal gland plasma membrane vesicles. The vesicles were prepared as in Fig. 1. The transport medium contained (in mM): mannitol 100, $Mg(NO_3)_2$, Tris-HEPES 20 (pH 7.6), NaCl 2, KCl 98 plus the diuretics in increasing concentrations from 10^{-5} to 10^{-3}M. The calculated inhibitory constants (K_i) for the diuretics are: bumetanide 8×10^{-5}M, piretanide 1×10^{-4}M, Bay g 2821 5×10^{-4}M and furosemide 5×10^{-4}M. The values represent the means of 2 to 3 experiments and are the average of uptake values measured at 1, 1.75 and 2.5 min. Prior to the transport experiment, the membrane vesicles were preincubated for 5 min at 0°C with 10^{-5}M of the diuretic.

transport. Similarly, in the TALH, the order of potency of the diuretics is identical but a two order of magnitude difference of sensitivity exists.

Several factors may play a role, first, the diuretics may act at additional sites in the cell thus explaining the higher diuretic sensitivity of the intact cell. Secondly, the transport experiments are performed at a low sodium concentration (1 mM compared to 280 mM in the perfusion fluid of the intact gland). This low sodium concentration may affect the affinity of the diuretics to the sodium-chloride cotransport system, similar to the effect of sodium on the affinity of the renal sodium-glucose cotransport system to phlorizen (5). Experiments such as binding studies of diuretics to isolated membranes could clarify this point. Supported in part by USPHS Grant AM 05841 and the Am. Heart Assn.-Maine Affiliate.

PIGMENTED SINUSOIDAL LINING CELLS IN ELASMOBRANCH LIVER

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Elasmobranch livers range in color from gray to a dark brownish-green. Histological examination of liver tissue fixed in 10% formalin and stained with hematoxylin and eosin revealed isolated brown pigmented cells which were situated within the region of the endothelial cells of the hepatic sinusoids, and were particularly prominent in livers from the small skate, Raja erinacea (Fig. 1A). Tissue was prepared for transmission electron microscopy by perfusing livers in the isolated state from 1 kg male skates as previously described (Reed, J.S., N.D. Smith, N. Tavoloni and J.L. Boyer, Bull. Mt. Desert Island Biol. Lab., 16:83-84, 1976). After a 30-minute perfusion with elasmobranch Ringers at 15°C, the livers were fixed by portal vein perfusion with Doyle's modification of Karnovsky's solution at

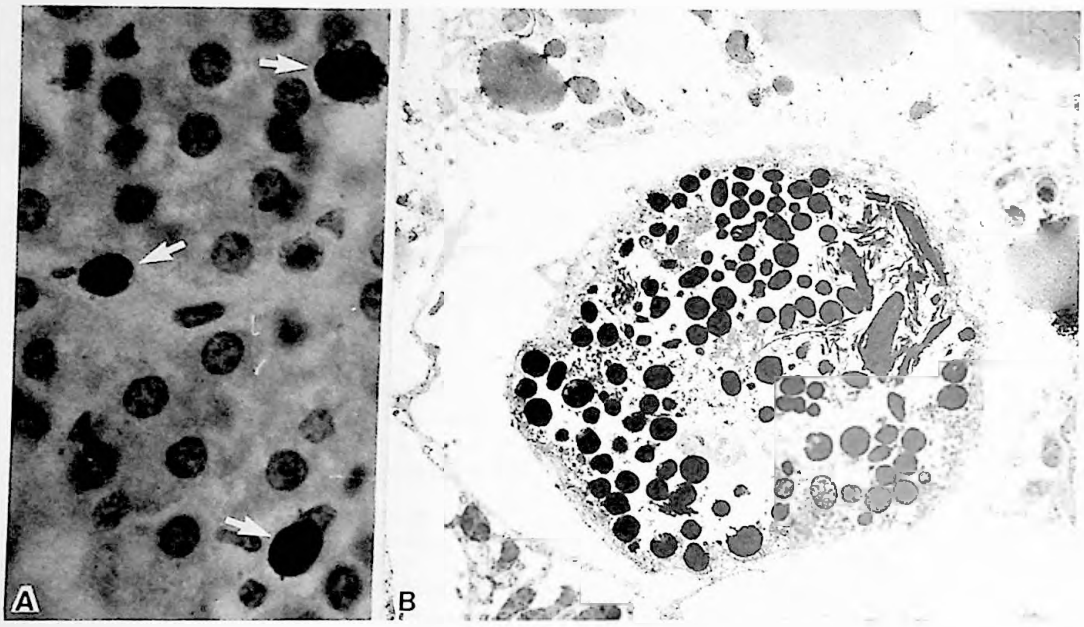

Figure 1. A. H & E stained section of skate liver. Magnification x 150. White arrows point to pigmented cells in hepatic sinusoids. B. Transmission electron micrograph of pigmented cell. Final magnification x 6500.

2.5 cm H_2O of hydrostatic pressure for approximately 10 minutes. Dissecting microscopic examination of raxor thin slices revealed pigmented cells localized predominantly to zone 3 of the hepatic lobule (central regions), a lobular distribution which was particularly notable in lightly pigmented livers. This zonal distribution was less prominent when darker livers containing a greater number of pigment cells were examined. Liver tissue was then examined by transmission electron microscopy after post fixation in osmium tetroxide (Fig. 1B). Occasionally, the cells were recessed into the space of Disse or the intracellular space between adjacent hepatocytes. Plasmalemma from pigment containing cells contacted adjacent endothelial cells or the surface of the hepatocytes. The cells were easily identified by the large number of small pigment granules which numbered more than 100 per cell section and which were densely osmophilic and occupied the majority of the cytoplasm. Characteristically, a large nucleus and prominent nucleoli were also observed. In occasional cells, an osmophilic crystalline material was also observed within the cytoplasm. Although the nature of the pigment and its origin are not clear, the morphologic characteristics of these cells closely resemble those of melanocytes observed in the skin of vertebrate species. Histologic examination of liver tissue from hagfish, fresh water eels, flounders, and goosefish fail to reveal pigmented sinusoidal lining cells. However, the darkly pigmented liver of the amphibian mudpuppy, Necturus, demonstrated dense sinusoidal infiltrates of similar pigmented cells. On the basis of these morphologic studies, we believe that pigment cells populate the sinusoidal lining cells of the hepatic sinusoid in elasmobranchs and in Necturus and account for the variable pigmented appearance of these livers. The origins of the pigment and the function of these pigment cells remains to be determined.

THE ISOLATED, PERFUSED HEAD OF THE LONG-HORNED SCULPIN: ADRENERGIC RECEPTORS CONTROLLING THE EFFECT OF EPINEPHRINE ON THE AFFERENT PERFUSION PRESSURE AND EFFERENT FLOW RATES

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Investigations of cardiorespiratory and osmoregulatory physiology of fishes has often involved the use of intact