will, of course, lead to irreversible deterioration of the fish. In the first ten minutes, the responses are remarkably similar. Since response to both hypoxia and hypercapnia is abolished by vagotomy or atropinization, the efferent pathway is the same. That the same central chemoreceptors are stimulated is only speculation. Hypoxemia may create local pH imbalance which in turn could stimulate receptors sensitive to high $CO_2$ tension. The lack of increase in resistance with hypoxia after parasympathectomy suggests there is no local constrictor effect of hypoxemia in the fish gill as there is in the vasculature of the mammalian lung.

From studies on the isolated perfused teleost gill it is known that acetylcholine increases the pressure drop across the gill (Comp. Biochem. Physiol., 12:127-142, 1964). Presumably blood is shunted away from respiratory lamellae through non-respiratory shunts to raise the pressure. Anatomical shunts have not been demonstrated in *S. acanthias*, but vagal innervation has been described for both basilar and distal arteries which deliver blood to and from the lamellae. If the increase in gill resistance in the spiny dogfish in response to hypoxia and hypercapnia is accompanied by a decrease in lamellar perfusion, then a reflex sensitive to post-gill blood gas changes might have great survival potential. Instead of losing oxygen as well as other solubles in the blood across the gills in a low $pO_2$ environment, the fish may simply decrease perfusion of the gill and the functional surface area until a more favorable environment is reached and $pO_2$ in the arterial blood increases. Studies quantitating the functional surface area of the gill during changes in gill resistance will be interesting.

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## PREPARATION AND ENZYMATIC PROPERTIES OF BRUSH BORDER AND BASAL-LATERAL MEMBRANES FROM FLOUNDER KIDNEY TUBULES

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Isolated flounder kidney tubules, first described by Forster in 1948 (Science, 108:65-67), have proved valuable for studying tubular transport, particularly active secretion of organic acids. Using rat kidney, recent studies with purified luminal and contraluminal plasma membranes have extended our understanding of the cellular and molecular mechanisms underlying transepithelial transport (Kinne, In: MTP International Review of Science, Kidney and Urinary Tract Physiology, Vol. II, edited by K. Thurau, Baltimore University Press, in press). Corresponding experiments with flounder kidney membranes are lacking and the present study was undertaken to evaluate procedures for purifying brush border (luminal) and basal-lateral (contraluminal) membranes from flounder tubules. Membranes separated by free-flow electrophoresis were compared with brush border membranes prepared by differential centrifugation. These membrane preparations were characterized by the presence of marker enzymes.

For the initial homogenate, kidneys of 6-8 flounder (*Pseudopleuronectes americanus*) were excised, chilled in ice-cold Forster's medium, and cut into small pieces with scissors. The tubules were released from the haematopoetic tissue by suction of the tissue suspension through a syringe and separated by low speed centrifugation. Freed tubules were homogenized to a 10 gm% homogenate in sucrose-Tris buffer (250 mM sucrose - 10 mM triethanolamine-HCl, pH 7.6 at 20°C). In accordance with the free-flow electrophoresis technique of Heidrich et al. (J. Cell Biol., 54:232-245, 1972)

crude plasma membranes were prepared from the initial homogenate and served as the starting material for separation of luminal (brush border) and contraluminal (basal-lateral) membranes. The conditions of the electrophoretic run were: 7±1°C, 90±10% volt/cm, 60 mA, electrophoresis buffer flow 3 ml/fraction. Using the differential centrifugation method of Booth and Kenny (Biochem. J., 142:575-581, 1974) an additional brush border fraction was prepared from the initial homogenate. This method is based on precipitation of basal-lateral plasma membranes as well as intracellular organelles by high concentrations of magnesium or calcium, the brush border membranes remaining in the supernatant after high-speed centrifugation. The marker enzymes for brush border membranes (alkaline phosphatase), basal-lateral plasma membranes ($Na^+$-$K^+$-ATPase), endoplasmic reticulum (glucose-6-phosphatase) and mitochondria (succinic dehydrogenase) were determined as described by Heidrich et al. (J. Cell Biol., 54:232-245, 1972). The activity of $Ca^{++}$-ATPase and $HCO_3^-$-ATPase were measured according to Kinne-Saffran and Kinne (J. Memb. Biol., 17:263-274, 1974 and Proc. Soc. Exptl. Biol. Med., 146:751-753, 1974). Protein was determined by the method of Lowry et al. (J. Biol. Chem., 193:265-275, 1951).

Figure 1 shows a typical distribution pattern for marker enzymes following free-flow electrophoresis. The distribution peaks for $Na^+$-$K^+$-ATPase and alkaline phosphatase were close, but consideration of the correlation coefficients between enzymes (r=0.82) suggests that the two maximum

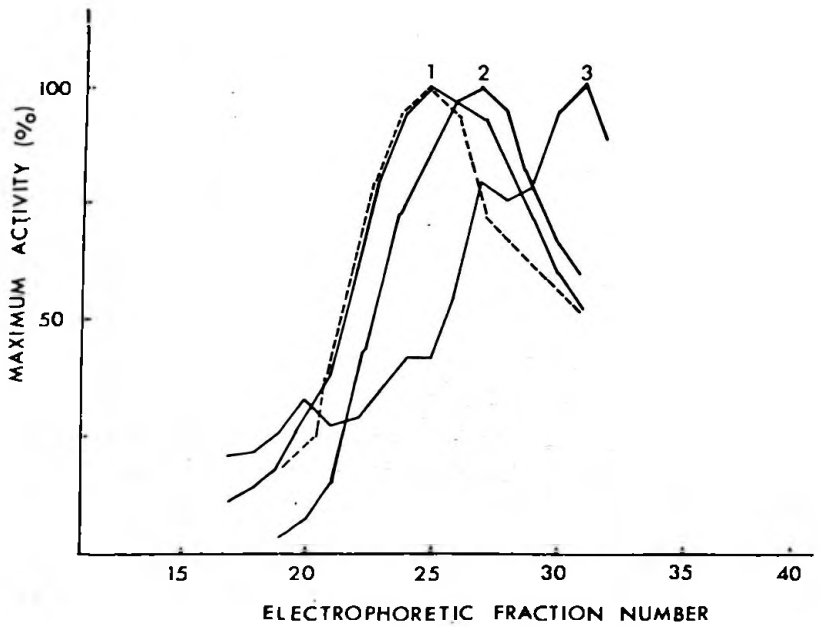


Figure 1. Distribution of marker enzymes after free-flow electrophoresis of a plasma membrane fraction from flounder kidney. Peak 1 is the distribution of $Na^+$-$K^+$-ATPase (the solid line) and $CA^{++}/Mg^{++}$-ATPase (the dashed line). Peak 2 represents the distribution of alkaline phosphatase and Peak 3 is $HCO_3^-$-ATPase. One representative experiment is given. The values are expressed as a percentage of the maximum activity (units/ml) of the individual enzymes found in each fraction obtained duirng electrophoresis.

activities were located in different electrophoretic fractions. The correlation coefficient between alkaline phosphatase and $Ca^{++}$-ATPase was likewise low (r=0.86), again indicating a separation of the basal-lateral from the brush border membranes. In contrast, the distribution of $Ca^{++}$-ATPase and $Na^{+}$-$K^{+}$-ATPase were very similar and highly correlated (r=0.97), demonstrating that both enzymes were located in the same basal-lateral fraction. Finally, $HCO_3^-$-ATPase did not appear to be related to any of the other enzymes determined; it had a separate peak and the correlation coefficient when compared with alkaline phosphatase was very low (r=0.63). This distribution is different from the rat kidney where $HCO_3^-$-ATPase seems integrally related to the brush border membrane (Kinne-Saffran and Kinne, Proc. Soc. Exptl. Biol. Med., 146:751-753, 1974). Attempts to improve the separation of the two membrane fractions by the addition of EDTA or calcium to the separation buffer failed. Experiments using the brush border membranes prepared by differential centrifugation as a starting material for free-flow electrophoresis, instead of the plasma membrane fraction, showed no further separation of the $Na^{+}$-$K^{+}$-ATPase and alkaline phosphatase.

Comparison of marker enzymes from the brush border membranes prepared by differential centrifugation with the membranes prepared by free-flow electrophoresis is shown in Table 1. Both brush border fractions showed a higher enrichment of alkaline phosphatase than of $Na^{+}$-$K^{+}$-ATPase,

TABLE I

ENRICHMENT OF MARKER ENZYMES IN THE BRUSH BORDER MEMBRANE FRACTIONS SEPARATED BY FREE FLOW ELECTROPHORESIS AND BY DIFFERENTIAL CENTRIFUGATION*

| Enzyme (Membrane location**) | Electrophoresis Brush Border† | Centrifugation Brush Border |
|---|---|---|
| Alkaline phosphatase (brush border) | 5.51 (2) | 8.7 (9) |
| $HCO_3^-$-ATPase (brush border) | n.d. | 1.78 (4) |
| $Na^{+}$-$K^{+}$-ATPase (basal-lateral) | 3.81 (2) | 2.8 (8) |
| $Ca^{++}$-ATPase (basal-lateral) | n.d. | 3.0 (3) |
| Succinic dehydrogenase (mitochondrial) | 0.44 (1) | 0 (5) |
| Glucose-6-phosphatase (endoplasmic reticulum) | n.d. | 1.7 (3) |

* Enrichment factor is defined as the ratio of the specific activity found in the fraction compared to the specific activity of the enzyme measured in the flounder kidney tubule homogenate. The number of experiments is given in brackets. n.d. = not determined. ** These enzyme locations are based on the assumption that the marker enzymes in flounder kidney are similar to those in rat kidney. † Combined fractions 26-30 from free electrophoresis.

whereas marker enzymes for endoplasmic reticulum and mitochondria were enriched less or were absent. This is especially true in the brush border membrane fraction prepared by differential centrifugation in which the specific activity of alkaline phosphatase was about nine times higher than in the homogenate, whereas $Na^{+}$-$K^{+}$-ATPase was enriched only 2.8 times. Therefore this brush border membrane

preparation was chosen to study the transport of glucose and p-aminohippurate (Eveloff et al., this bulletin) because of the ease of preparation and high enrichment of alkaline phosphatase.

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## CALCIUM EFFECT ON SUGAR TRANSPORT IN TEASED RENAL TUBULES OF THE WINTER FLOUNDER *Pseudopleuronectes americanus*

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Previous studies have demonstrated a multiplicity of sugar transport systems in the renal tubule cells of several species. These transport systems are characterized not only by differing stereospecificities but also by differing cellular locations. This situation complicates any analysis of transport phenomena since the experimentally determined tissue sugar accumulation values may represent an integration of transport via two or more pathways. The use of teased flounder tubules circumvents to some degree these problems of localization since some evidence has been presented suggesting that sugar transport in this preparation reflects events taking place primarily at the antiluminal face (Kleinzeller and McAvoy, J. Gen. Physiol., 62:169-184, 1973). The experiments presented herein give further support to the validity of this view.

Trump (Federation Proceedings, 30:22-41, 1971) observed that incubation of teased flounder tubules in a calcium-free saline had two notable effects: (1) a marked cellular swelling and, (2) a weakened integrity of the "tight" intercellular junction (as evidenced by the inability of these tubules to concentrate chlorphenol red within their luminal space). If this ionic manipulation of the medium does indeed lead to markedly increased permeability of the intercellular junction, then sugars (or other compounds of low molecular weight) should gain access to that luminal space from which they are normally excluded in the teased tubule preparation. Accordingly, incubation of tubules in a Ca-free medium should give these tubules transport properties demonstrable in a system where the luminal face of the cell is freely accessible to the studied solute, e.g., clearance studies (Pritchard and Kleinzeller, Am. J. Physiol., 231:603-607, 1976). In particular, the brush-border localized, active transport system for methyl-α-D-glucoside (α-me-Glc), known to be affected by a Na-dependent, highly phlorizin-sensitive mechanism, should become apparent. The cellular uptake of D-galactose (Gal) should also be increased by the absence of Ca.

The present experiments involved incubation (or preincubation) of teased tubules in normal (1.4 mM Ca) or Ca-free saline and measurement of the tissue accumulation of three model sugars: α-meGlc, Gal, and 2-deoxy-D-galactose (2-dGal). Agents known to inhibit active sugar transport, i.e., phlorizin and ouabain, were also tested for their effects. Table 1 shows the effect of Ca-free medium on the accumulation of these sugars. α-MeGlc and Gal, which have been shown to be reabsorbed from the lumen in clearance studies (Pritchard and Kleinzeller, Am. J. Physiol., 231: 603-607, 1976), show greater accumulation in tubules incubated in Ca-free saline. The tissue to medium ratio (T/M) of α-meGlc rose well over 1.0, indicating active transport under these conditions. 2-dGal, however, is not reabsorbed from the lumen (Pritchard and Kleinzeller, Bull. MDIBL, in press, 1976) and was not found to accumulate to any greater degree in tubules incubated in Ca-free medium.

Phlorizin reduced the space of all three sugars to values close to that of the extracellular space. A further effect of Ca-free medium was to increase the water and Na content and to decrease