membranes was estimated by extrapolation of the uptake data to infinite osmolarity and was found to be negligible. Using equilibrium uptake values for D-glucose and PAH (60 and 7 pmoles/mg protein, respectively) and assuming that intravesicular and extravesicular concentrations are equal (0.11 mM and 10.8 μM, respectively) it is calculated that 1 mg of membrane protein represented an intravesicular space of 0.5 μl for both solutes. Thus, PAH and D-glucose are transported into the same vesicular space.

The results described above provide evidence for carrier-mediated transport of PAH as well as D-glucose into plasma membrane vesicles prepared from flounder kidney tubules. Since this membrane preparation contains predominantly brush border membranes, it is tempting to conclude that both transport systems are located in the brush border membrane. This conclusion is supported by combined studies on sugar transport in the intact flounder kidney and teased kidney tubules (Pritchard and Kleinzeller, Am. J. Physiol., 231:603-607, 1976) in which energy-dependent phloridzin-sensitive transport of D-glucose-like sugars was demonstrated at the luminal side of the tbular cells. Definitive cellular localization of the PAH transport system (s) in flounder tubules, however, requires improved separation of luminal from contraluminal plasma membranes.

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DISTRIBUTION OF ENZYMES IN MEMBRANES OF RAT KIDNEY CORTEX, WITH PARTICULAR REFERENCE TO CARBONIC ANHYDRASE

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Carbonic anhydrase is present in the microsomal membranes of the rat and human kidney cortex as well as in the soluble fraction (Maren and Ellison, Mol. Pharm., 3:503, 1967; McKinley and Whitney, BBA, 445:781, 1976). These preparations represent plasma membranes, endoplasmic reticulum and ribosomes. Data are lacking, however, on the specific localization of the enzyme in the renal cortical cell. The present experiments were undertaken to determine whether carbonic anhydrase is present in the total plasma membrane and in a brush border membrane preparation from rat kidney cortex.

Male Sprague-Dawley rats weighing 300-600 gm were killed by decapitation, the kidneys were removed and the cortex excised after perfusion through the renal artery with cold sucrose-Tris buffer (0.25 M sucrose - 0.01 M Tris OH, pH 7.6 with HCl) to remove contaminating red blood cells. Homogenized cortical tissue plasma membranes were isolated by the method of Fitzpatrick et al. (J. Biol. Chem., 244: 3561, 1969) and brush border membranes were prepared using a modification of the method of Booth and Kenney (Biochem. J., 142:575, 1974). In this modification, 10 mM $CaCl_2$ was added to the renal cortical homogenate and aggregated subcellular organelles and basal-lateral membranes were removed by low-speed centrifugation. The supernatant fraction was centrifuged at 15,000 x g to yield a pellet rich in brush border membranes. Prior to measuring the enzyme activities, all preparations were freeze dried and stored at -20°C. Marker enzymes for cell membranes were assayed as follows: alkaline phosphatase, a plasma membrane enzyme (Monod et al., Nature New Biol., 240:126, 1972), $(Na^+ + K^+)$-ATPase a basal-lateral membrane marker (Kinne et al., Pflugers Arch., 329:191, 1971), HCO_3^--ATPase, a brush border membrane enzyme (Kinne-Saffron and Kinne, Proc. Soc. Exptl. Biol. Med., 146:751, 1974), Ca^{++}-ATPase, a plasma membrane enzyme (Parkinson and Radde, Biochem. Biophys. Acta, 242:238, 1976) and succinic dehydrogenase, a mitochondrial enzyme (Gibbs and Reimer, Proc. Soc. Exptl. Biol. Med., 119:470, 1965). Carbonic anhydrase was measured by the method of Maren (J. Pharm. Expt. Therap., 130:26, 1960). Protein determinations were carried out according to Lowry et al. (J. Biol. Chem., 193:265, 1951). The techniques

are such that brush border enzymes are included in the determination of enzymes in total plasma membranes, but that the brush border determinations exclude the baso-lateral membranes.

Table 1 shows the specific activities and relative enrichments of the plasma membrane and brush border enzymes. Alkaline phosphatase $(Na^+ + K^+)$-ATPase, HCO_3^-=ATPase and Ca^{++}-ATPase were enriched in the plasma membrane fraction 8.3, 5.4, 3 and 4 times, respectively. There was no mitochondrial contamination in the plasma membrane fraction. The brush border membrane preparation showed a high (31) enrichment of alkaline phosphatase, and a correspondingly low (0.8) enrichment of $(Na^+ + K^+)$-ATPase. HCO_3^--ATPase and Ca^{++}-ATPase were enriched 3 and 2 fold, respectively. Mitochondrial contamination was very low in the brush border membrane preparation.

TABLE 1

Activity of Enzymes in Rat Kidney Cortex Homogenate, Plasma Membranes and Brush Border

	Specific Activity*			Enrichment**	
	Homogenate (n=8)	Plasma Membrane (n=4)	Brush Border (n=4)	Plasma Membrane	Brush Border
Alkaline phosphatase	4.6 ±.8	38 ±6	142 ±40	8.3	31
$(Na^+ + K^+)$-ATPase	2.6 ±.4	14 ±.6	2 ±1	5.4	0.8
HCO_3^--ATPase	3.1 ±.3	9 ±1.3	8 ±1	3	3
Ca^{++}-ATPase	8.5 ±1.2	37 ±2	20 ±3	4	2
Carbonic anhydrase†	23900 ±2650 (4550)	7900 490	5100 950	0.33	0.21
Succinic dehydrogenase	0.2 ±.07	n.d.	0.004 ±.001		0.02

*Specific activity is defined as μmoles substrate converted/mg protein · hr at 37°C. Values are means ± standard error. n.d. = not detectable.

**Enrichment factor is the ratio of specific activities of enzymes in these fractions to that of homogenate.

†Values at 0°C. Enzyme units for carbonic anhydrase activity are also shown (in parentheses) and expressed as (uncatalyzed time - catalyzed time)/catalyzed time per gram of tissue. In the method used the volume is 1 ml and barbital buffer (4 mM) at pH 7.9 is used. On this scale of unitage rat red cells are about 30,000 units/ml.

The carbonic anhydrase concentration (per mg protein) in the plasma membranes was 33% of that in the renal cortical homogenate. In the brush border the equivalent number is 21%. Care was taken, as in the experiments cited above, to ensure that contamination from the soluble fraction was avoided. Carbonic anhydrase is a water soluble enzyme classically assoc iated with the supernatant fraction of secretory cells; the nature of its attachment to the membranes is presently unknown. The present data show that it is not in the same quantitative category as enzymes that are enriched in membranes. However, because of its very high turnover number, the presence of even small amounts can be significant.

The carbonic anhydrase activities in the homogenate, membranes and brush border were 50% inhibited with 10^{-8} M methazolamide or acetazolamide and fully inhibited at 10^{-6} M, suggesting the presence of a single "high activity" isozyme in all the fractions.

In epithelia which are capable of acidifying their mucosal fluid, e.g., the gastric mucosa and kidney, the serosal fluid is simultaneously alkalinized. This process is equivalent to the splitting of a water molecule and a transfer of H^+ ions to the mucosal side of the epithelial cell and a concomitant transfer of OH^- ions to the serosal side. This process has been shown to occur in the proximal tubules of rats (Filho and Malnic, Pflugers Arch., 363:211, 1976). Contraluminal carbonic anhydrase catalyzes the reaction $OH^- + CO_2$, buffering rapidly the high OH^- and providing HCO_3^- as a counter ion to sodium for reabsorption. At brush border there is no analogous reaction and there appears to be a lower concentration of enzyme here than in total membrane. The function of carbonic anhydrase at brush border has been supposed to be the rapid dehydration of luminal H_2CO_3 (Rector, in The Kidney, ed. by B. M. Brenner and F. C. Rector, Vol. I, 1976, Saunders); although if H_2CO_3 is diffusible, there is no compelling reason why this reaction cannot be mediated by the very high concentration of enzyme in the cytosol (Maren, Can. J. Physiol. Pharm., 52:104, 1974).

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THE TRANSPORT OF 3-O-METHYL-D-GLUCOSE FROM BLOOD TO BRAIN AND OCULAR FLUIDS IN THE DOGFISH

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The movements of various monosaccharides in many biological systems are facilitated by so-called carrier-mediated processes. There is strong evidence that such mechanisms operate across brain capillary endothelium (e.g., Crone, J. Physiol., 181:103, 1965) and choroid plexus epithelium (e.g., Fishman, Am. J. Physiol., 206:836, 1964) of mammals. As part of an ongoing study of blood-brain-cerebrospinal fluid (CSF) transfer mechanisms in *Squalus acanthias*, an examination of 3-O-methyl-D-glucose (3 MG) transport from blood to brain and CSF was begun. In these same experiments, the distribution of this sugar from blood to aqueous and vitreous humors and skeletal muscle was also studied.

Method. Dogfish weighing from 1.5-3.0 kg were used. Solutions containing both an ^3H-labeled material (3 MG) and ^{14}C-labeled compounds (3 MG or urea) were injected intramuscularly in the same fish. Sometimes the ^3H- and ^{14}C-compounds were injected simultaneously; in other cases, the two labeled materials were administered at different times (the latter procedure allowed the study of 3-O-methyl-D-glucose's distribution for two different time periods in the same animal). The experimental durations were 5, 10, 15, 30, and 60 minutes plus 2, 3, and 4 hours. At 10-12 times during the course of each experiment, samples of blood were obtained. Except for the few seconds when blood samples were drawn, the dogfish swam freely in large sea water filled tanks.

At the end of the experimental period, the animals were removed from the water and quickly decapitated. Immediately thereafter a sample of CSF was taken, and both eyes plus the brain removed. Samples of aqueous humor were aspirated from each eye; subsequently both eyes were frozen in preparation for regional sampling of the vitreous humor. Pieces of brain (medulla) and skeletal muscle were obtained for analysis. Samples of fluid from the anterior, central, and posterior regions of the