(Bull. MDIBL 19:70-72, 1979). This increased rate of entry of sodium is blocked by furosemide or bumetanide. High-affinity ouabain binding (at 10^{-9}M and 10^{-8}M) was therefore measured in slices incubated with and without theophylline and cyclic AMP in the presence of furosemide (10^{-4}M) and bumetanide (10^{-5}M). Other experiments were carried out in which sodium was replaced by lithium, or chloride was replaced by nitrate (Table 1) maneuvers that also inhibit secretion by interfering with sodium chloride cotransport. Theophylline and cyclic AMP stimulated ouabain binding even in the presence of furosemide or bumetanide and whether or not sodium or chloride were present in the incubation solution.

From these experiments we conclude that theophylline and cyclic AMP alter the binding of ouabain to rectal gland cells and therefore probably alter the characteristics of membrane Na-K-ATPase. This appears to be the result of an increase in the affinity for ouabain at high-affinity sites on cell membranes although changes in the number of sites cannot be excluded. An increase in secretory activity is not a necessary step for the increase in ouabain binding produced by theophylline and cyclic AMP. Cyclic AMP appears to initiate a series of cellular events that alter membrane Na-K-ATPase directly rather than as a secondary result of ion movements in the course of stimulated secretion.

KINETICS OF ^{35}S-SULFOBROMOPHTHALEIN UPTAKE FROM ALBUMIN SOLUTIONS IN ELASMOBRANCH LIVER

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Recent studies in mammalian systems have suggested that the hepatic uptake of sulfobromophthalein (BSP), bilirubin, and long chain fatty acids may involve a saturable interaction of the albumin with receptor sites on the cell surface (Science 211:1048, 1981). Elasmobranchs offer an opportunity to investigate the nature of these receptor sites, for while they take up and excrete BSP, these marine species lack serum albumin and by inference a specifically evolved albumin receptor.

Uptake kinetics were determined at 15°C by using a multiple steady-state, single-pass perfused liver method as previously described (ibid). Male skates (Raja erinacea, 0.75-1.2 kg) were maintained for up to five days in aerated tanks before livers were isolated by the method of Reed et al., (Am. J. Physiol., in press, 1982). After a 30-min equilibration period during which livers were perfused with recirculating oxygenated Elasmobranch Ringer solution, livers were perfused with a sequence of single-pass test solutions in which either the concentration of ^{35}S-BSP was systematically varied at a fixed albumin concentration, or the concentrations of both ^{35}S-BSP and bovine serum albumin were varied at a fixed (1:50) BSP:albumin molar ratio ($\bar{\nu}$). Uptake was studied at albumin concentrations of 0.05, 0.25 and 0.75% using $\bar{\nu}$ values up to 3; for a fixed $\bar{\nu}$ of 1:50, albumin concentrations were 0.15-1.5%. Five to 6 BSP solutions were each perfused for 3-min in one liver, and effluent samples were obtained for scintillation counting after steady-state uptake was achieved. Each test solution was followed by a 5-min perfusion of a similar albumin solution lacking BSP, which rapidly reduced effluent radioactivity to negligible levels. The net steady-state uptake rate for each solution was calculated as the product of the first-pass extraction fraction, the flow rate per gram of liver and the total BSP concentration. Data were analyzed by non-linear, least-squares computer curve fitting and the results for replicate livers averaged. The equilibrium concentration of free BSP was estimated for each solution by computer analysis using published binding constants (J. Clin. Invest. 45:281, 1966).

Figure 1 relates BSP uptake velocity (solid line) to total BSP concentration over the range 4-112 μM for each of two albumin concentrations. At 0.75% albumin (panel A) saturation of uptake is evident with linear double-reciprocal plots (not shown). The apparent K_m and V_{max} are shown in Table 1. At 0.25% albumin

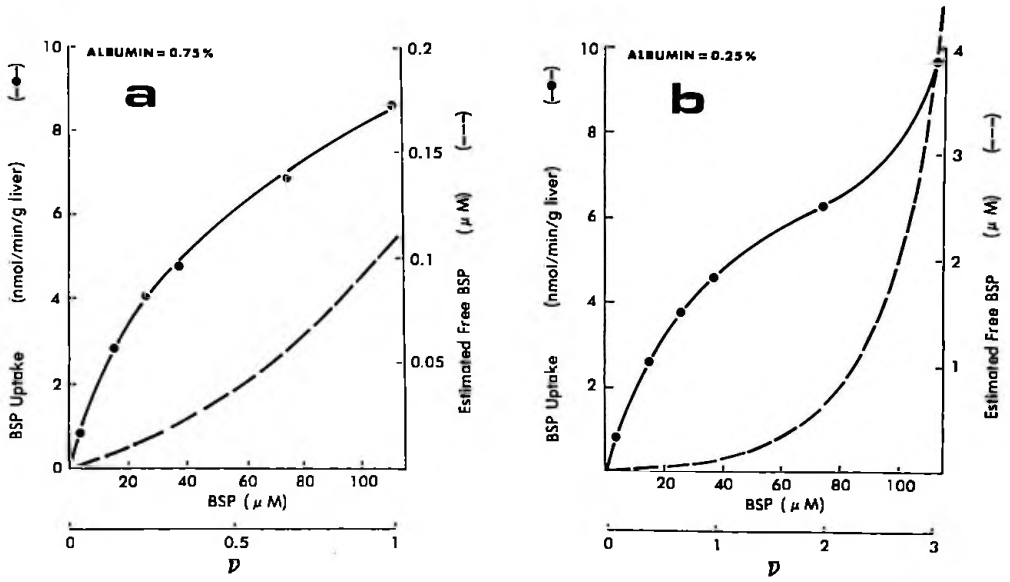

Figure 1.--Linear plot of uptake velocity (solid lines) versus total BSP concentration for fixed albumin concentrations of 0.75% (A) and 0.25% (B). The estimated equilibrium free BSP concentration for each BSP:albumin molar ratio ($\bar{\nu}$) is provided for comparison (broken lines).

Table 1.--BSP Uptake Kinetics for Fixed Albumin Concentrations

Albumin	Apparent K_m*	Apparent V_{max}*	Range	Livers Studied
(%)	(μM, Mean $\pm$ SEM)	(nmol/min/g liver)	BSP:Albumin Ratio ($\bar{\nu}$)	
0.75	47.6	11.6	0.1 - 1.0	2
0.25	49.0 $\pm$ 10.4	10.5 $\pm$ 1.7	0.1 - 2.0	5
0.05	38.0 $\pm$ 6.4	17.9 $\pm$ 4.5	0.1 - 2.0	3

*All p values >0.1.

(panel B), results are nearly identical to those at 95%, except at the highest BSP concentration, where the observed velocity significantly exceeds that predicted by the rest of the plot ($p < 0.05$). This acceleration in velocity may be due to the much greater fraction of free BSP that occurs at these higher BSP-albumin ratios ($\bar{\nu}$) (Fig. 1, lower abscissa). These observations are consistent with a model in which the uptake velocity is determined primarily bh the bound BSP fraction at lower molar ratios, while at molar ratios greater than 2, the free fraction becomes large enough to contribute significantly to uptake. Table 1 lists apparent K_m and V_{max} values for BSP uptake at three different albumin concentrations, using only data for which $\bar{\nu} \leq 2$.

These results indicate that like the mammal, hepatic BSP uptake in elasmobranchs is a saturable process with kinetic constants essentially independent of the albumin concentration (Table 1), suggesting a saturable uptake event which is intrinsic to the liver.

Figure 2 shows the result of simultaneous variation of both the BSP and albumin at a fixed 1:50 molar ratio, where concentrations of free BSP are negligible. Saturation kinetics are seen with an apparent K_m of 1.45 $\pm$ 0.29 μM BSP or 73.5 $\pm$ 14.5 μM albumin and a V_{max} of 0.700 $\pm$ 0.199 nmol/min/g liver ($n = 4$). The uptake velocity appears independent of the equilibrium free BSP concentration (Fig. 2A, broken line), again suggesting that uptake

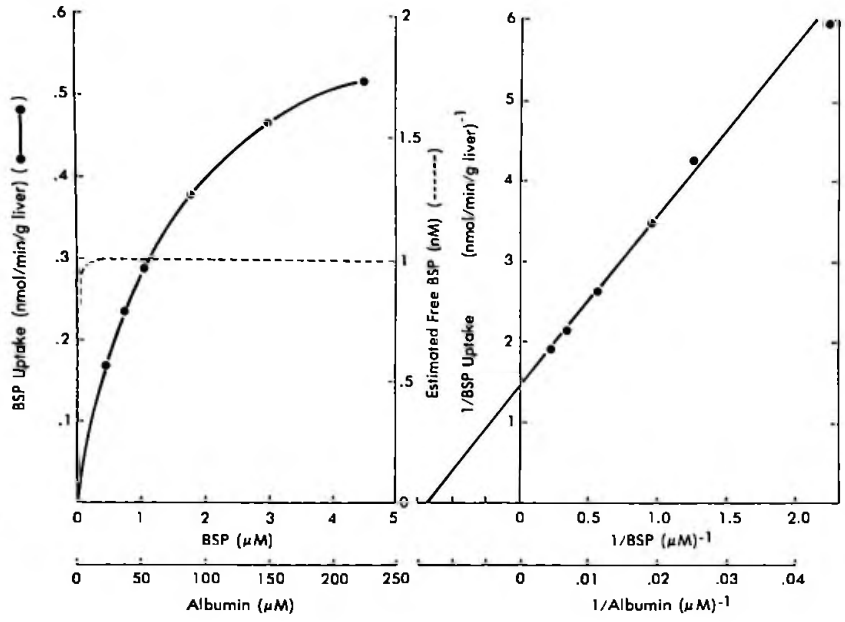

Figure 2.--Uptake velocity is shown for different concentrations of the 1:50 BSP:albumin complex (solid lines) on linear (A) and double reciprocal (B) coordinates. The estimated equilibrium free BSP concentration is shown for comparison in panel A (broken line).

depends primarily on the bound BSP fraction (assuming that binding, equilibrium is maintained within the sinusoid). The much lower V_{max} observed in these experiments suggests that when free BSP is minimized, a different uptake step is rate limiting and that this step may involve a saturable interaction of albumin with the liver cell surface. The low $\bar{\nu}$ (1:50) was selected for this experiment so that the uptake velocity would always be low with respect to the intrinsic V_{max}, and yet the albumin concentration would be large enough to test for a saturable interaction. Increasing the molar ratio to 1:20 did not affect the apparent K_m when calculations were based on albumin concentration, however the apparent K_m calculated from the BSP concentration increased approximately four-fold ($n = 2$). Under these conditions, the interaction of the complex with the cell surface and not the intrinsic uptake step appears to be rate limiting, and appears to depend on the albumin and not the BSP concentration. Similar uptake kinetics have been observed in the rat (Science 211:104, 1981) suggesting that uptake of albumin-bound substances may involve receptor sites for albumin on the cell surface. Since the elasmobranchs lack albumin, one would not expect to find a highly specific receptor for bovine albumin. Bovine albumin may therefore have a binding affinity for some less specialized component of the plasma membrane which might be similar in both rats and skates. Saturation of uptake at higher concentrations of the BSP–albumin complexes might result from depletion of the available binding sites caused by albumin binding, or from saturation of the available surface of the membrane with albumin molecules.

In summary, these studies demonstrate that the multiple steady state single-pass technique can be utilized to assess the mechanisms of organic anion extraction in the isolated perfused skate liver. The results suggest that BSP uptake is a saturable process that is primarily determined by the binding of a BSP–albumin complex rather than by free BSP under the conditions of these experiments.

DIFFERENCES IN THE DETERMINANTS OF ^{35}S-SULFOBROMOPHTHALEIN (^{35}S-BSP) AND ^{14}C-TAUROCHOLATE (^{14}C-Tc) CLEARANCE IN THE ISOLATED PERFUSED SKATE LIVER

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Preliminary studies from this laboratory have demonstrated that the kinetics of hepatic organic anion clearance