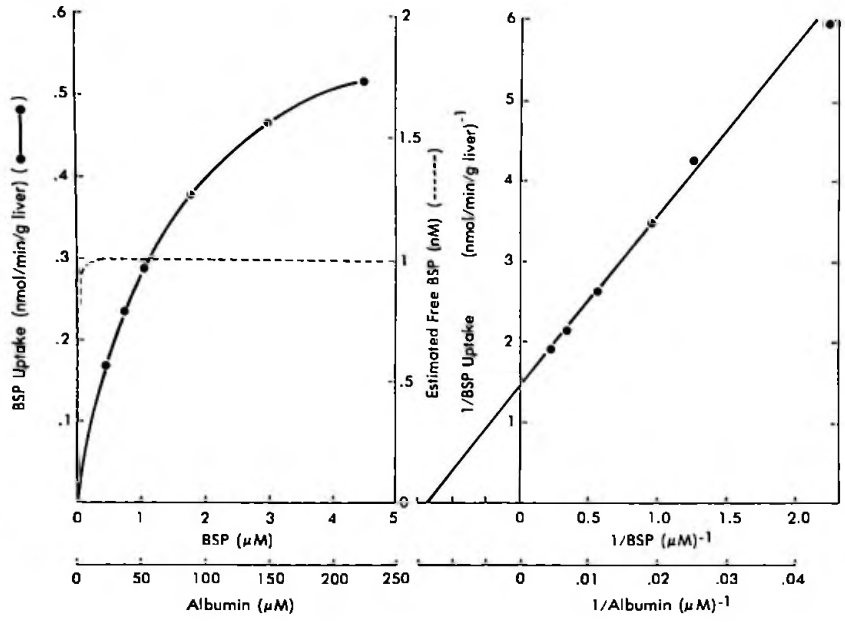

Figure 2.--Uptake velocity is shown for different concentrations of the 1:50 BSP:albumin complex (solid lines) on linear (A) and double reciprocal (B) coordinates. The estimated equilibrium free BSP concentration is shown for comparison in panel A (broken line).

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

(BSP and taurocholate) can be assessed by using a single pass method in isolated perfused skate livers (see Weisiger et al., Bull. MDIBL, 1981). In the present experiments, we compare the effects of temperature, sodium concentration, and organic anion inhibition on the kinetics of BSP and taurocholate (Tc) extraction. In addition, values of V_{max} for BSP and Tc are predicted from estimates of the free and albumin-bound ligan and compared to experimental values, in order to examine the relative importance of the protein bound fraction as a determinant of their observed rates of extraction.

Values for single pass clearance of ^{35}S-BSP and ^{14}C-Tc were determined in isolated perfused skate livers using 4-6 concentrations of ligand over the range of 3.7 μM - 75 μM, and a fixed concentration of bovine serum albumin (0.25%) as previously described (Weisiger et al., Bull. MDIBL, 1981). Temperature dependence was assessed for each ligand by comparing extraction kinetics at control (8-15°C) and high (20-25°C) temperatures, using one liver preparation for both temperature ranges in each experiment. To determine sodium dependence, the hepatic clearance of each concentration of either ^{14}C-Tc or ^{35}S-BSP was examined in both control and low sodium media in the same liver preparation (choline chloride was substituted for sodium chloride in the Elasmobranch Ringer's at 15°C).

A low sodium wash period of 5 min preceded a 3 min infusion of the labelled anion, also in low sodium media. Identical concentrations of ligand in the control medium were alternately infused for each concentration of ligand. To study inhibition effects, two sets of experiments were performed: 1) BSP inhibition of ^{14}C-Tc uptake and 2) Tc inhibition of ^{35}S-BSP. In each experiment, clearance was assessed over the same range and concentrations of ligand used previously, initially with no inhibitor, followed by inhibitor concentrations of 37 μM and 75 μM. Proportions of bound and free ligand in 0.25% bovine serum albumin for the concentrations used were estimated as described (by serial approximation, see first abstract) using Scatchard binding data of Baker and Bradley (JCI 545:281, 1966) for Tc.

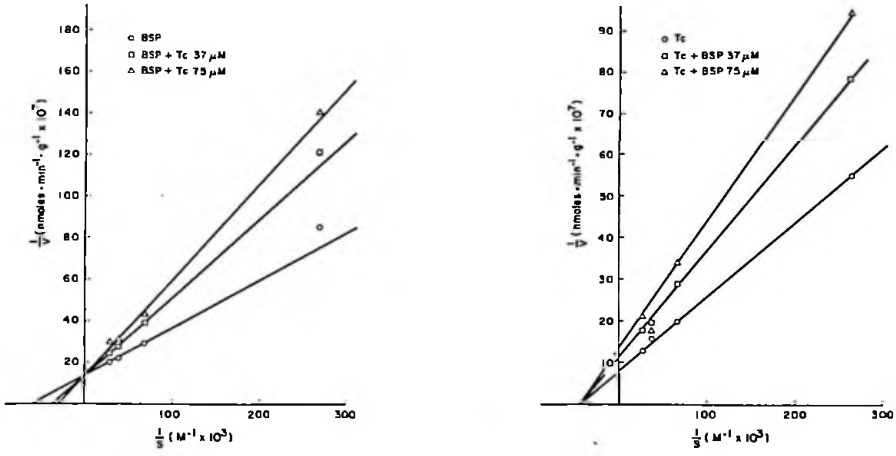

Figure 1.--(left)-Double reciprocal plots of taurocholate clearance in the isolated perfused skate liver (open circles) and in the presence of 37 μM BSP (open boxes) and 75 μM BSP (open triangles). Three to four different concentrations of taurocholate were utilized to generate each line. All points were generated from the same perfused liver. The kinetics suggest non-competitive inhibition of BSP on taurocholate clearance.

Figure 2.--(right)-Double reciprocal plots of a representative experiment of sulfobromophthalein clearance in the isolated perfused skate liver (open circles) and in the presence of 37 μM taurocholate (open boxes) and 75 μM taurocholate (open triangles). Three to four concentrations of BSP were utilized to generate each line, and all points were obtained from the same skate liver. Taurocholate competitively inhibits the hepatic clearance of BSP in this species.

Results indicate that the hepatic clearance (apparent V_{max}) of ^{14}C-Tc and ^{35}S-BSP approximately doubled with a 10° rise in temperature, yielding Q_{10} ratios of 1.87 for Tc and 1.92 for BSP.

During sodium substitution experiments, perfusate sodium concentration was reduced from control values of 280 mEq to 22.8 ± 1.8 mEq, which did not affect the apparent V_{max} for the clearance of ^{35}S-BSP. V_{max} for uptake of ^{14}C-Tc was consistently reduced when choline was substituted for sodium, though significance was not achieved. However, when ^{14}C-Tc in a low sodium solution was followed by an isotope free control wash, ^{14}C-Tc in the effluent fell transiently but then increased, suggesting that transport of ^{14}C-Tc from liver to perfusate (counter-transport) was stimulated by sodium, a phenomenon that was not observed with ^{35}S-BSP.

Tc competitively inhibited ^{35}S-BSP clearance (Fig. 1) whereas BSP demonstrated non-competitive inhibition of ^{14}C-Tc clearance (Figure 2).

For ^{35}S-BSP, the observed V_{max} corresponded closely to values predicted using the bound concentration and differed greatly from estimated values when the free concentration was used. In contrast, the V_{max} observed for ^{14}C-Tc was greater than values predicted for either fraction alone, suggesting that Tc clearance is dependent on both the bound and free fractions. These results are summarized in Table 1.

Table 1.--Kinetics of ^{35}S-BSP and ^{14}C-Tc Extraction, with Predictions Based on Albumin Bound and Free Fractions at 0.25% Albumin

		PREDICTED	
V_{max} (nmoles min^{-1} g^{-1} liver)	Observed	Bound	Free
Taurocholate (n-4)*	25.1 ± 4.9	5.93 ± .95	18.9 ± 3.8
BSP (n=8)	7.50 ± 1.85	8.04 ± 2.18	0.026 ± .008
Km (μM)			
Taurocholate (n=4)	51.0 ± 11.4	11.7 ± 1.8	38.7 ± 9.3
BSP (n=8)	37.2 ± 13.1	38.9 ±13.2	0.128 ± .041

*n = number of livers studied

We conclude from these studies that 1) The temperature dependence of hepatic organic anion clearance is consistent with a carrier mediated transport process. 2) The hepatic clearance of Tc, but not BSP, appears to be partially dependent on sodium in the perfusion medium. 3) Competitive inhibition of ^{35}S-BSP clearance by Tc suggests a shared pathway for these two organic anions at some point in the transport process in this primitive vertebrate species. The pathways do not appear identical, however, as suggested by non-competitive inhibition of ^{14}C-Tc by BSP, a possible differential dependence on sodium, and the greater importance of albumin bound BSP than bound Tc in the uptake of these anions.