cellular K^+ gradient, especially during long term depolarization caused by the cellular secretory process. Clearly this hypothesis requires rigorous measurements of ionic selectivity to Cl^- and K^+ at the two states. We have no evidence at present that such a change of selecting in fact occurs. We should like to thank Drs. Silva and Epstein for providing us with the isolated cells and for many helpful discussions.

MECHANISMS OF TAUROCHOLATE UPTAKE IN THE SKATE LIVER EFFECTS OF SODIUM ION AND ALBUMIN

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INTRODUCTION--Studies in mammals indicate that Na^+ is required for Taurocholate (Tc) transport into the hepatocyte, a process which may represent coupled transport of Tc^- and Na^+ via a membrane associated carrier (Boyer, Physiol. Rev. 60:303-326, 1980). More recently, serum albumin has been shown to facilitate Tc uptake in mammalian liver (Forker, J. Clin. Invest. 67:1517-1522, 1981), possibly through recognition of an albumin-anion complex at a specific receptor. The latter might show Na^+ dependence either by Na^+ -dependent recognition of the complex of by an effect on the complex association constant under conditions where the complex concentration is limiting.

Our previous studies utilizing single pass clearances in isolated perfused skate livers indicate that hepatic Tc uptake from albumin solutions is temperature dependent, saturable and competitive with bromsulphathalein, supporting a carrier mediated process (Zacks, et al Bull. MDIBL 21:110, 1981). In the present study, we use this system to determine if Tc uptake is Na^+ dependent, and whether this effect can be attributed to a Na^+ dependent membrane carrier for free Tc anion, to changes in Tc affinity for albumin, or to Na^+ dependent membrane recognition of the albumin-Tc complex.

METHODS--Equilibrium Dialysis: To determine whether Na^+ affects Tc binding to albumin, association constants (Ka) were obtained in triplicate by equilibrium dialysis in elasmobranch ringers solution at 37°C prepared with either NaCl (high Nat, 270 mEq) or choline Cl (low Na^+, 20 mEq) media. Dialysis bags, containing 37.5 μM fatty acid free Bovine Serum Albumin (BSA and 37.5 μM ^{14}C-NaTc were immersed in either high or low Na^+ ringers solution and allquots were removed at 12 h intervals until equilibrium was reached at 48 h. Ka was calculated as (CPM in/CPM out) (1/BSA) at equilibrium. Ka's were also measured in NaCl ringers at 15°C to determine effects of low temperatures. Ka values were compared to the published value of 1.22×10^7 measured in a mammalian medium at 37°C. (Green, et al BBA 231:250-252, 1971). Using measured Ka's, the concentration of bound and free Tc were calculated at equilibrium for each of the combinations of Tc and BSA used in skate liver perfusion experiments, to determine the influence of albumin on hepatic uptake rates of Tc.

Tc-Clearance Studies: The hepatic clearances of Tc were determined using a multiple steady state, single pass method adapted for the perfused skate liver as previously described (Weisiger, et al Bull MDIBL 2:108, 1981) using three concentrations of ^{3}H-NaTc (3.73, 26.1 and 74.5 μM). Either Na^+ or choline chloride elasmobranch Ringers was used to assess the effect of Na^+. To assess the effect of albumin, livers were perfused with either 0.25% BSA (7 studies) or 2.0% BSA (5 studies).

^{14}C-Tc-BSA solutions in NaCl or choline or chloride were alternately infused for 3 min. each, to establish a steady extraction rate for Tc. A 15 min wash infusion of the same medium without Tc preceeded each study. Immediately prior to perfusion with the unlabelled wash solution, four 1.5 ml samples of hepatic venous effluent were obtained every 15 seconds and aliquots analyzed by liquid scintillation counting for unlabelled wash solution, four 1.5 ml samples of hepatic venous effluent were obtained every 15" and aliquots analyzed by liquid scintillation counting for determination of Tc extraction.

Additional hepatic venous samples were taken at 1,3,5,10 and 15 min after the unlabelled wash. Infusion

was begun, to monitor a return of effluent CPM to baseline. Each subsequent extraction measurement was corrected for their respective baseline values in the hepatic effluent. Net steady state uptake (J) was calculated as $J = (1 - \frac{CPM_o}{CPM_i})\ Q[Tc]$ where Q is perfusion rate per gram wet liver and (Tc) is initial total Tc concentration. Differences in Tc uptake rate in Na^+ and choline media were compared by paired t-tests. Low sodium experiments were also performed (3 studies) using LiCl instead of choline Cl.

Tc-clearance rates, obtained in 0.25% and 2.0% BSA, were compared to determine if variation in the concentration of the BSA-Tc complex would effect rates of Tc uptake (the latter increased the bound Tc fraction from 21% to 78%).

RESULTS: The equilibrium dialysis experiments (Table 1) indicate that the BSA-Tc binding constant in elasmobranch ringer at 15°C is not significantly different from that at 37°C. A similar constant was observed in both Na^+ and choline media. All determinations approximated the published value of 1.22×10^4 measured at 37°C in mammalian ringers.

Table 1.--Equilibrium Dialysis Determined Association Constants of Bovine Serum Albumin and Taurocholate in Elasmobranch Ringer's Solution ($\times 10^{-4}$), for All Determinations n = 3

	-NaCl- 15°C	37°C	-Choline Cl- 15°C
EXPT I	1.2 ± .19	--	1.0 ± .22
EXPT II	1.42 ± .23	1.50 ± .09	---

In the perfusion experiments, a significant decrease in net uptake rate was consistantly observed when Na^+ was replaced with choline, except when Tc/BSA ratios exceeded 1.0 (Table 2). Reintroduction of Na^+ after perfusion

Table 2.--Taurocholate (Tc) uptake rates $\times 10^{-8}$ moles $min^{-1} g^{-1}$ liver

3.73 μM Taurocholate	Na^+	Choline	Δ	
0.25% Albumin	.19 ± .04	.14 ± .03	+ .48 ± .03	P < .01
molar ratio = 0.1				n = 7
2.0% Albumin	.15 ± .07	.11 ± .06	+ .03 ± .02	P < .01
molar ratio = 0.013	NS	NS		n = 5
26.1 μM Taurocholate				
0.25% Albumin	.93 ± .15	.68 ± .18	+ .26 ± .16	P < .02
molar ratio = 0.7				n = 6
2.0% Albumin	.74 ± .31	.62 ± .38	+ .12 ± .09	P < .05
molar ratio = .087	NS	NS		n = 5
74.5 μM Taurocholate				
0.25% Albumin	1.68 ± .39	1.47 ±	+ .21 ± .43	NS
molar ratio = 2.0				n = 6
2.0% Albumin	1.58 ± .77	1.32 ± .83	+ .26 ± .10	P < .01
molar ratio = 0.25	NS	NS		n = 5

with choline transiently increased effluent ^{3}H-Tc concentration, a phenomenon that was never observed with reintroduction of choline. When lithium replaced Na^{+}, a decrease in uptake rate was observed at 3.73 μM Tc in 0.25% BSA but not consistantly at higher Tc concentrations. Increasing BSA concentration from 0.25% to 2.0% did not significantly change the observed uptake rate even though this approximately tripled estimated equilibrium concentrations of bound Tc at each of the 3 Tc concentrations used (Table 3).

Table 3.--Taurocholate uptake rates in skate liver perfused with 0.25% and 2.0% Bovine serum albumin

3.73 μM Taurocholate % BSA	Estimated Bound conc.	$\times 10^{-8}$ moles min^{-1} g^{-1} liver uptake rate
0.25%	1.14 μM	.191 $\pm$ .04
2.0%	2.92 μM	.145 $\pm$ .07
26.1 μM Taurocholate		
0.25%	7.04 μM	.943 $\pm$ .15
2.0%	20.2 μM	.740 $\pm$ .31
74.5 μM Taurocholate		
0.25%	15.6	1.58 $\pm$ .39
2.0%	55.7	1.58 $\pm$ .77

DISCUSSION--Because the Ka for Tc and BSA was not changed with choline substitution, the effect of Na^{+} on Tc clearance could not be attributed to changes in Tc-BSA binding. Measurement of Ka in elasmobranch ringers at 15°C and 37°C gave values nearly identical to published values at 37°C in mammalian ringers, indicating that neither the temperatures nor ionic strengths tested affect this equilibrium. By exclusion, the observed Na– dependence must be attributed to either a membrane carrier for free Tc or a membrane-Tc-Albumin interaction. Because rates did not increase when the bound concentration was increased by increasing BSA (as would be expected if complex concentration facilitated uptake), complex recognition by the sinusoidal membrane seems unlikely in contrast to studies in perfused rat liver. However, a significant increase in Tc clearance rates was observed as total Tc concentration was increased, at fixed BSA concentration. Indicating that the free or total Tc, rather than the albumin bound fraction is a major determinant of the net rate of uptake. These results are most consistent with a Na^{+} facilitated coupled transport of free Tc across hepatic sinusoidal membrane as the anion becomes available by dissociation from albumin. Supported by USPHS Grant #AM25636.

EVALUATION OF A SULFATED BILE ALCOHOL IN THE BILE OF THE SMALL SKATE (RAJA ERINACEA)

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Hepatologists in general agree today that active bile salt secretion plays an important role in bile formation in man, dog, rat and the guinea pig, presumably by producing an osmotic pressure gradient within the canaliculi that promotes the passive movement of water and diffusible solute from sinusoidal blood to the canalicular lumen. To what extent such a mechanism may be similarly operative in lower vertebrates is unknown. The bile acids in the Cyclostomes and Elasmobranchs appear to be largely sulphated bile alcohols that differ with respect to their physiochemical properties from the bile salts commonly found in mammalian bile (Haslewood, GAD, The Biological Importance of Bile Salts, North Holland, Oxford, 1978). Just how these differences may affect the contribution of