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SODIUM-LINKED TRANSPORT OF ETHACRYNIC ACID BY RAT LIVER: POSSIBLE SIGNIFICANCE FOR CHOLERETIC ACTION

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Abstract—Uptake, biotransformation and biliary excretion of ethacrynic acid was investigated in the isolated rat liver and related to the choleretic effect of the drug. Ethacrynic acid transport from sinusoidal extracellular space into liver cells is mediated by a saturable, energy-dependent, and partially Na^+-dependent transport mechanism. Extracellular sodium stimulates translocation of ethacrynic acid across the sinusoidal membrane by increasing the maximal velocity from 0.47 to 0.64 μmoles.min^{-1}.g liver^{-1} without any major effect on the substrate affinity of the carrier. Within the cell, ethacrynic acid is rapidly and almost completely metabolized to its glutathione derivative which, in turn, is excreted into bile canaliculi by a saturable transport system. Canalicular excretion of metabolized ethacrynic acid is the rate-limiting step in hepatic transport of the choleretic drug (V_{max} 0.15 μmoles.min^{-1}.g liver^{-1}). Both translocation steps are accompanied by an increase in transmembrane sodium fluxes. At the sinusoidal site, co-transport of Na^+ with ethacrynic acid and/or inhibition of (Na^+-K^+)-ATPase might be responsible for the net increase in intracellular Na^+ as observed in liver slices. Excretion of intracellular Na^+ into bile canaliculi is enhanced during canalicular transport of the ethacrynic acid glutathione adduct. The transepithelial Na^+ net movement induced by these events might be underlying the stimulatory effect of ethacrynic acid on bile secretion.

The diuretic drug ethacrynic acid exerts a profound choleretic effect in various species, including rat [1, 2], guinea pig, rabbit, dog and sheep [3]. Czok and Schulze [4] showed that ethacrynic acid undergoes biotransformation in the liver before secretion into bile. These authors suggested that stimulation of bile output might be due to an osmotic effect of excreted metabolites. The same conclusion was reached by Chenderovitch *et al.* [5], while Knodell [6] concludes than an alteration in hepatic electrolyte excretion might be involved in the choleretic action of ethacrynic acid.

It is well known that ethacrynic acid inhibits sodium extrusion from kidney proximal tubule cells either by its interference with an electroneutral sodium pump and/or by inhibition of the (Na^+-K^+)-ATPase [7]. Thus, an effect on cellular Na^+ transport has to be considered as a possible cause of ethacrynic acid stimulation of basal, i.e. bile salt-independent bile secretion [8]. In fact, modulation of transepithelial Na^+ flux has been linked to the choleretic action of ouabain [9], cholate or taurocholate [10]. These substances are thought to augment sinusoidal-to-canalicular Na^+ transport [11], either by inhibition of Na^+ efflux at the sinusoidal pole of the hepatocyte via interaction with the (Na^+-K^+)-ATPase or by increasing Na^+ influx through Na^+-coupled active entry from the sinusoidal space [9, 10, 12].

The current study concerns the question of whether stimulation of basal bile flow by ethacrynic acid is accounted for by a possible association between hepatic transport of the choleretic drug and transmembrane Na^+ fluxes.

MATERIALS AND METHODS

Liver perfusions. Male Sprague–Dawley rats [strain Him:OFA (SPF), 180–200 g] were obtained from the Forschungsinstitut für Versuchstierzucht, Himberg, Austria, and used as liver donors (liver weight 9–11 g). The surgical procedure for isolation of the liver has been described elsewhere [13]. The livers were perfused in a hemoglobin-free recirculating perfusion system as described by Scholz [14]. The standard perfusion medium was Krebs–Henseleit bicarbonate buffer (pH 7.4) supplemented with 5.05 mM glucose. In some experiments a 'sodium-free' perfusion medium was used. This solution was obtained by isosmolar substitution of NaCl by choline chloride and of $NaHCO_3$ by Tris–HCl (pH 7.4).

In all experiments, the isolated liver was pre-perfused with standard medium for 30 min to allow recovery from the anoxic state during surgery. Perfusion was then switched to a second independent recirculating system containing an appropriate concentration of radio-ethacrynic acid in 100 ml medium. Binding of ethacrynic acid to the perfusion system was found negligible when checked in control perfusions in absence of the isolated organ.

Bile flow was monitored by an automatic drop counter (H. Pichler, J. Graf and M. Peterlik, unpublished) which allowed continuous recording of secretion rates.

$^{24}Na^+$ efflux into perfusate and bile was measured by preloading isolated livers with 1.0 mCi $^{24}Na^+$ per 100 ml perfusion medium for 60 min, followed by perfusion with non-recirculating 'cold' medium.

After 20 min, ethacrynic acid was infused into the portal tubing for 10 min to obtain a perfusate concentration of 0.5 mM. Perfusate was collected at 1.0 min intervals while the bile collection period was 2.0 min. Perfusate and bile samples were counted in a Beckman Gamma 800 counter.

Reagents. Ethacrynic acid and radiolabelled ethacrynic acid ([phenoxyacetic-2-^{14}C]-ethacrynic acid, 5 mCi/mmole) was generously donated by Merck, Sharp & Dohme, Rahway, NJ. The purity of the compound was checked by thin-layer chromatography (see later). All radioactivity was found to migrate in a single peak.

Carbonyl cyanide *m*-chlorophenyl hydrazone was purchased from Sigma Chem. Co., St. Louis, MO.

Fractionation of perfused livers. At the end of some perfusion experiments, the liver was perfused for 3 min with ethacrynic acid-free perfusion medium (either standard or sodium-free). The liver was then homogenized in 1.15% KCl. Subcellular fractions were prepared by differential centrifugation using a standard protocol [15].

Thin-layer chromatography (t.l.c.). Bile samples (150 μl) were dried in a rotatory evaporator at 37°. The solid was extracted with 0.2 ml 50% aqueous ethanol. Portions (50 μl) of the solution were applied to a t.l.c. plate. Perfusate samples (2.0 ml) were treated with 4.0 ml ethanol, and the precipitate was removed by centrifugation. The supernatant fraction was evaporated to dryness and extracted with 200 μl of 50% aqueous ethanol. Eight millilitres of the cytoplasmic fraction was mixed with 24 ml ethanol. The mixture was allowed to stand overnight at 4° and was then centrifuged. The supernatant fraction was evaporated and the resulting solid material extracted by shaking with 500 μl 50% ethanol for 30 min at room temperature. In all, 94–97 per cent of radioactivity could be recovered from perfusate, liver or bile samples by these extraction procedures.

The adsorbent was silica gel G (Merck, Darmsstadt, F.R.G.). The t.l.c. plates were developed with toluene–acetic acid–water (50/50/5, v/v/v). Radioactivity was determined either directly on the plate with a gas-flow thin-layer scanner (Berthold, Wildbad, F.R.G.) or by liquid scintillation counting after extraction of the adsorbent with 50% ethanol. Ethacrynic acid and its glutathione adduct were identified by co-chromatography with the authentic substances. The latter was synthesized as described by Klaassen and Fitzgerald [16].

Liver slices were prepared with a Stadie–Riggs microtome and incubated under continuous gassing (O_2/Co_2, 95/5%) in Krebs–Henseleit bicarbonate buffer supplemented with 2.0 mM sodium pyruvate as described by Haylett and Jenkinson [17]. After incubation, tissue specimens were frozen in liquid nitrogen and transferred into pre-weighed vials, containing 1.0 ml distilled water. After mild overnight shaking, Na^+ was determined in the supernatant fraction by flame photometry. Extracellular space was determined with [^{14}C-carboxy]inulin (Radiochemical Centre, Amersham, U.K.). Average intracellular Na^+ and K^+ concentration of controls in three experiments were 36.3 ± 2.1 and 91.9 ± 3.2 m-equiv./kg wet wt, respectively. To eliminate the influence of interexperimental variation, data were calculated as percentages of the mean of the corresponding control group.

Liquid scintillation counting. Ten millilitres of a toluene-based scintillation cocktail with adequate amounts of a sample solubilizer (Biosolv, Beckman) were added to samples of tissue, perfusate, bile and subcellular fractions. Radioactivity was determined in a Beckman LS 230 liquid scintillation counter. Quench correction was by external standardization.

RESULTS

Choleretic action of ethacrynic acid. As detailed in a previous report [2], ethacrynic acid stimulates bile secretion in a dose-dependent manner. Figure 1 illustrates the typical time-course of ethacrynic acid-induced choleresis at three different dose levels. Characteristically, the onset of choleresis is very rapid and peak secretion is reached within a few minutes (Table 1). Bile flow then steadily declines to pre-drug values. As shown in Table 1 and exemplified in Fig. 1 (lower part), biliary excretion of radiolabelled ethacrynic acid lags behind elevated secretion. Maximal radioactivity in bile is observed distinctly after the maximum of secretion and, likewise, biliary excretion persists even after return of bile flow to basal values. This discrepancy between stimulation of bile production and biliary excretion of the choleretic drug is even more obvious when excretion rates vs increment in bile flow are plotted

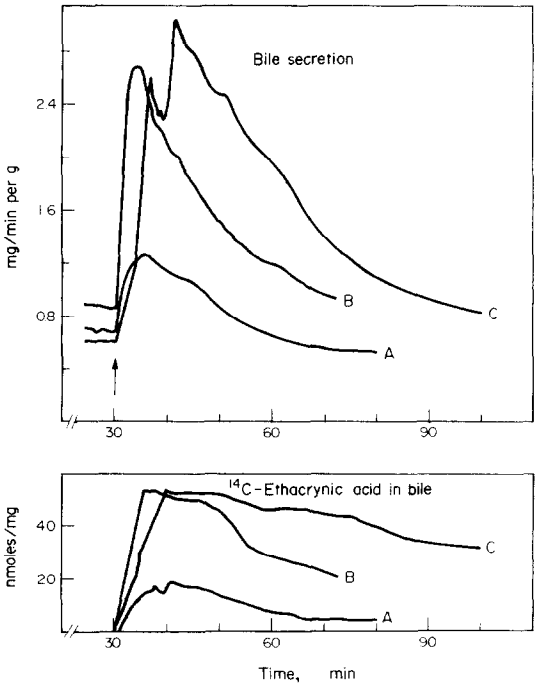

Fig. 1. Choleretic effect of ethacrynic acid in isolated rat liver (upper part) and biliary excretion of ethacrynic acid (metabolites) (lower part). Initial drug concentrations in 100 ml perfusion medium were: A, 8.3 μmoles; B, 33 μmoles; and C, 82.5 μmoles. Arrow indicates switching of perfusion system to ethacrynic acid-containing medium. Each curve represents a single experiment. Additional data are given in Table 1.

Table 1. Time-course of ethacrynic acid-induced bile secretion and biliary extretion of [^{14}C]-ethacrynic acid (equivalents)

Initial perfusate concentration of ethacrynic acid (μmoles/ml)	Bile secretion		Biliary excretion of [^{14}C]-ethacrynic acid metabolites	
	Maximum	Decline to half maximal values	Maximum	Decline to half maximal values
	(min after addition of ethacrynic acid)			
0.033	3	7	5	9
	3	7	4	10
0.083	6	17	11	27
	6	18	8	22
0.165	4	16	6	25
	4	13	8	18
0.333	5	20	7	35
	4	16	7	25
0.495	4	22	7	30
	4	25	4	35
0.825	8	33	10	>60
	8	28	12	35

for the 15-min interval following the addition of the drug to the perfusion system. During this time, the glutathione derivative of ethacrynic acid is the only metabolite excreted into bile (see later). Figure 2 reveals that at the onset of choleresis effective stimulation of bile flow occurs at relatively low excretion rates. This suggests that some mechanism in addition to a choleretic action of the excreted metabolite is responsible for ethacrynic acid induction of bile formation.

Uptake of ethacrynic acid by perfused liver (Fig. 3, Table 2). In the case of ouabain- or bile acid-induced choleresis, the fraction of bile flow not caused by osmotic activity of the excreted substances was traced to an increase in transepithelial sodium flux resulting from coupled Na^+ uptake from the perfusion medium [9, 10, 18]. To test whether this also holds true for ethacrynic acid, uptake kinetics and the

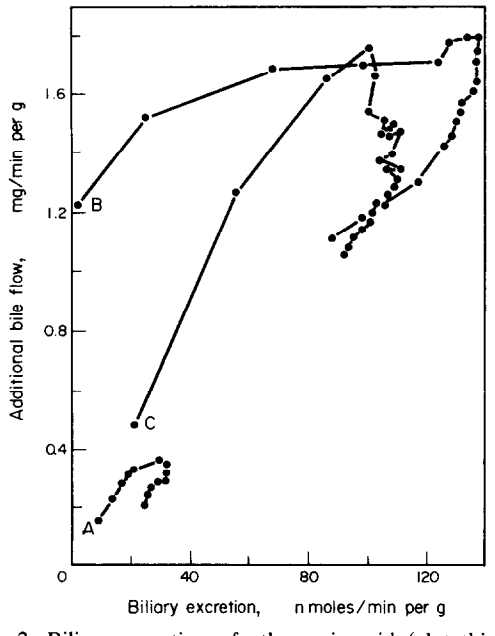

Fig. 2. Biliary excretion of ethacrynic acid (glutathione adduct) vs additional bile flow. Initial ethacrynic acid concentrations as in Fig. 1. Curves were calculated from data shown in Fig. 1.

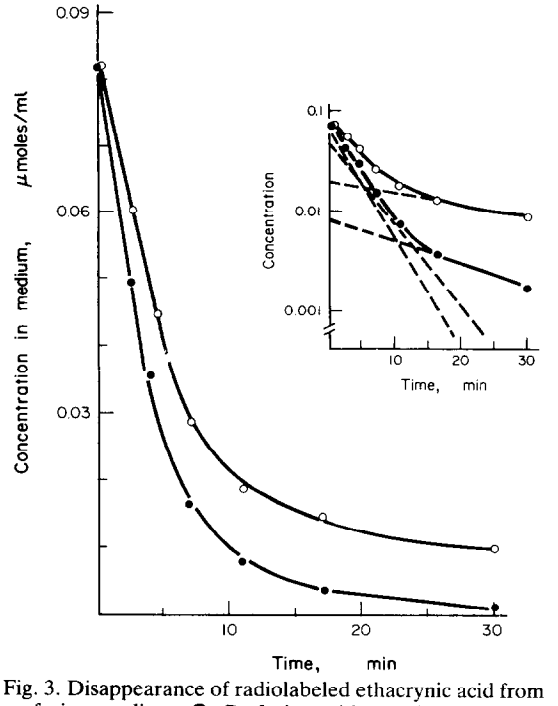

Fig. 3. Disappearance of radiolabeled ethacrynic acid from perfusion medium. ●, Perfusion with standard medium, liver weight 9.0 g; ○, perfusion with sodium-free medium, liver weight 9.5 g. Initial perfusate concentration was 0.083 μmoles/ml medium. Inset: Semi-logarithmic plot of elimination kinetics. Calculated velocities of rapid and slow transfer are shown by dotted lines. Additional data are given in Table 2.

effect of Na^+ thereon were studied in a concentration range from 0.033 to 0.825 μmoles/ml medium. Figure 3 illustrates the typical pattern of ethacrynic acid clearance from the perfusion system. In all experiments, linear initial uptake was observed during a short time interval (not exceeding 4 min). Representation of the elimination kinetics in a semilogarithmic plot reveals that disappearance of the drug from the perfusion system is a composite of two phases: initially, a rapid transfer of ethacrynic acid into the isolated liver is observed, followed by a second phase of slow decline of perfusate drug levels. Omission of Na^+ from the perfusion medium does not change this general pattern of uptake but obviously decelerates both transport processes as evidenced by consistently lower values of the respective elimination constants in the sodium-free state (Table 2). As reported earlier [11], sodium-free perfusion leads to immediate cessation of bile flow.

Involvement of a carrier-mediated step in the rapid phase of ethacrynic acid transfer is suggested from nonlinear dependence of initial transport rates on drug concentration (Fig. 4, uper part) at both normal and zero perfusate sodium. When a term of diffusional uptake is deducted (see below), both curves approach the typical rectangular hyperbola of Michaelis–Menten kinetics and can be linearized for calculation of the kinetic constants. Sodium accelerates the translocation of ethacrynic acid by increasing the maximal velocity (from 0.47 to 0.64 μmoles. $min^{-1}.g\ liver^{-1}$), while K_m remains virtually unaffected (0.26 and 0.13 mM, respectively). Due to the rapid clearance of ethacrynic acid from the perfusion medium substrate concentrations changed rapidly even during the short time interval used for determination of initial velocities. Therefore, calculation of kinetic constants had to be based on initial (zero time) perfusate concentrations and might, thus, deviate slightly from theoretical values.

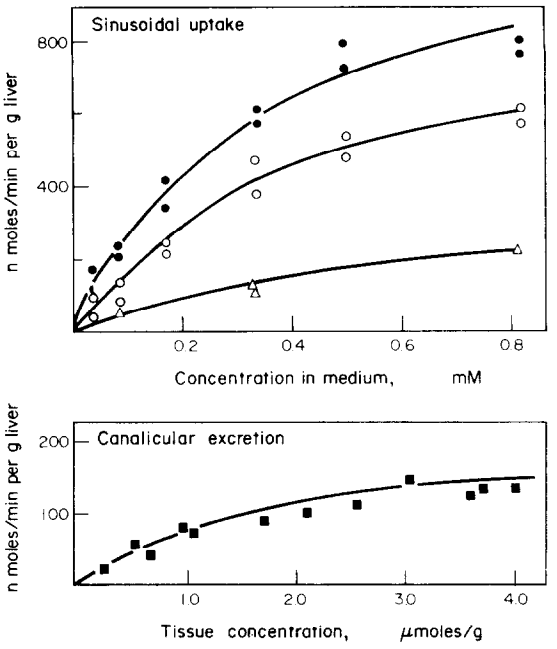

Fig. 4. Kinetics of rapid component of ethacrynic acid uptake by isolated liver (upper part) and of biliary excretion of the glutathione derivative (lower part). Uptake rates were calculated from the initial part of the elimination curve as far as it can be considered linear (up to 4 min, cf. Fig. 3). Correction of the data obtained was by subtraction of the rate of slow-phase ethacrynic acid transfer which had been calculated from the respective elimination constant (k_2, Table 2), thereby excluding its contribution to measured uptake rates. ●, Perfusion with standard medium; ○, perfusion with sodium-free medium; △, metabolic inhibition by 50 μM mClCCP; ■, maximal canalicular excretion rate (the corresponding tissue concentration was calculated from disappearance of ethacrynic acid from perfusate and was corrected for prior excretion of the glutathione adduct into bile).

Table 2. Elimination constants (k) of biphasic ethacrynic acid clearance from perfusion system*

Initial perfusate concentration of ethacrynic acid (μmoles/ml)	k_1 ($g^{-1}.min^{-1}$)		k_2 ($g^{-1}.min^{-1}$)	
	Standard medium	Na^+-free medium	Standard medium	Na^+-free medium
0.033	0.037	0.025	0.0030	0.0023
	0.050	0.019	0.0050	0.0020
0.083	0.022	0.012	0.0050	0.0031
	0.025	0.009	0.0042	0.0033
0.165	0.017	0.011	0.0039	0.0008
	0.022	0.010	0.0054	0.0010
0.333	0.015	0.011	0.0019	0.0006
	0.014	0.008	0.0019	0.0005
0.495	0.012	0.008	0.0008	0.0003
	0.013	0.007	0.0006	0.0003
0.825	0.006	0.004	0.0007	0.0002
	0.006	0.004	0.0008	0.0002

* Subscripts 1 and 2 refer to rapid and slow phase, respectively, of ethacrynic acid uptake by isolated liver (cf. Fig. 3, inset). k_1 and k_2 are calculated as slopes of the theoretical disappearance curves (dotted lines, Fig. 3, inset) using the fomula $c = c_o e^{-kt}$. Measured values of rapid phase ethacrynic acid transfer were corrected for the slow component by a 'peeling off' method.

Table 3. Distribution of radiolabeled ethacrynic acid between perfusion medium, liver and bile after 10 min perfusion*

Perfusion medium	Initial concentration in perfusion medium (μmoles/ml)	[^{14}C]-Ethacrynic acid equivalent			Bile/liver concentration ratio
		Perfusate (μmoles/ml)	Liver (μmoles/g)	Bile (μmoles/ml)	
Standard	0.033	0.001 (0.0005–0.0015)	0.09 (0.07–0.11)	18.4 (16.6–20.2)	204
	0.083	0.014 (0.008–0.018)	0.32 (0.28–0.37)	29.5 (23.3–34.8)	92
	0.165	0.041 (0.032–0.051)	0.75 (0.71–0.77)	54.6 (46.5–66.8)	73
	0.333	0.120 (0.105–0.146)	2.11 (2.09–2.13)	57.8 (55.3–60.2)	27
	0.495	0.131 (0.107–0.173)	2.43 (2.20–2.82)	50.0 (47.5–53.1)	21
	0.825	0.302 (0.253–0.320)	3.14 (2.54–3.73)	54.1 (47.6–60.5)	17
Na^+-free	0.033	0.003 (0.002–0.004)	0.29 (0.26–0.32)	—	—
	0.083	0.022 (0.019–0.025)	0.58 (0.52–0.63)	—	—
	0.165	0.047 (0.045–0.050)	1.54 (1.43–1.57)	—	—
	0.333	0.126 (0.103–0.156)	2.79 (2.66–2.88)	—	—
	0.495	0.231 (0.200–0.262)	3.44 (3.38–3.51)	—	—
	0.825	0.432 (0.430–0.433)	3.47 (3.24–3.70)	—	—

* Data are means (range of variation) from two or three perfusion experiments. Liver content of ethacrynic acid equivalents was calculated from dissappearance of radiolabel from perfusate and corrected for biliary excretion in perfusion experiments with standard medium.

The deviation of transport kinetics from a rectangular hyperbola (Fig. 4) suggested the involvement of an additional term in the rapid phase of ethacrynic acid transport into liver cells, probably reflecting a diffusional pathway. Ethacrynic acid uptake was, therefore, measured during metabolic inhibition by 50 μM carbonyl cyanide *m*-chlorophenyl hydrazone (mClCCP), a potent uncoupler of oxidative phosphorylation. Ethacrynic acid entry into the isolated liver (Fig. 4) was markedly reduced. Furthermore, separation of the disappearance curve into two phases was no longer detectable (not shown).

These results allow the conclusion that uptake of ethacrynic acid into the liver is mediated mainly by an energy-dependent, partially Na^+-sensitive active transfer step whereas contribution from a diffusional pathway is relatively small.

* Association of radioactivity with particulate fractions is certainly overestimated as data are not corrected for cross-contamination by adhering soluble phase.

Metabolism, intracellular distribution and canalicular transport of ethacrynic acid. As it is well known that ethacrynic acid undergoes biotransformation in the liver [4, 16], we attempted to assess the role of ethacrynic acid metabolism in sinusoidal-to-canalicular transport. The data presented in Table 3 suggest that this transport consists of two subsequent concentrative transfer steps, namely, from perfusate to liver and, most obvious, from liver to bile. The retarding influence of sodium-free perfusion is reflected by higher perfusate levels of ethacrynic acid. Furthermore, the lack of biliary excretion due to cholestasis in the Na^+-free state results in pronounced intrahepatic accumulation of radiolabel.

Table 4 shows that accumulated radioactivity is confined mainly to the soluble phase of liver cells after 10 min perfusion.* This was not changed by perfusion of the isolated liver with Na^+-free medium.

The cytoplasmic fraction was analysed for ethacrynic acid metabolites by thin-layer chromatography and compared to the composition of perfusate and

Table 4. Intracellular distribution of ethacrynic acid (equivalents) after 10 min perfusion*

Cell fraction	Initial ethacrynic acid concentration in perfusion medium (μmoles/ml)			
	Standard medium		Na^+-free medium	
	0.165	0.495	0.165	0.495
Nuclear	12.6	—	8.8	—
Mitochondrial	5.4	—	1.0	—
Lysosomal	1.7	—	1.0	—
Microsomal	7.6	—	0.9	—
Total particulate fractions	27.3	26.7	11.7	29.6
Cytoplasm	81.9	74.3	90.5	74.4
Recovery	109.2	101.0	102.2	104.0

* Data are expressed as percentage of total dpm in liver homogenate. Each column represents a single experiment.

Table 5. Relative distribution (%) of ethacrynic acid (EA) and its glutathione derivative (EA-GSH) in perfusate, liver and bile after 10 min perfusion*

Perfusion medium	Initial EA concentration (μmoles/ml)	Perfusate		Cytoplasm		Bile	
		EA	EA-GSH	EA	EA-GSH	EA	EA-GSH
Standard medium	0.495	100	0	7	93	6	94
	0.825	100	0	5	95	9	91
Na^+-free medium	0.495	100	0	5	95	—	—
	0.825	100	0	6	94	—	—

* Samples were analysed by thin-layer chromatography as described under Materials and Methods. Sum of integrator readings of t.l.c. scanner was set to 100 per cent. A single experiment was performed for every EA concentration given.

bile (Table 5). Strikingly, ethacrynic acid taken up from the perfusion medium was rapidly and almost completely converted into its glutathione adduct. A similar relative concentration of the two compounds is observed in bile. Rapid conversion of ethacrynic acid into its metabolite was also observed in the Na^+-free state. It was obvious that, even at the highest ethacrynic acid concentration employed, the glutathione pool of the isolated liver is by no means depleted during the time interval under investigation. The glutathione adduct was never found in the perfusion medium. In contrast to the canalicular site, there is no efficient transport mechanism for the outward movement of this metabolite at the sinusoidal site of the plasma membrane.

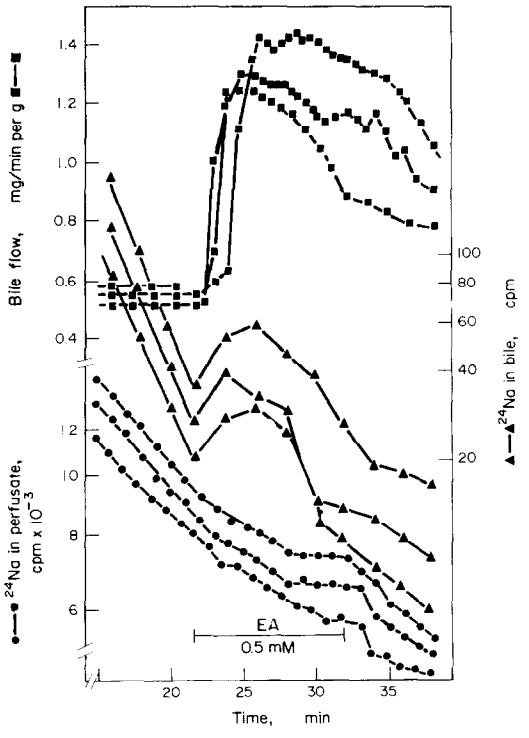

Fig. 5. Changes in transmembrane sodium fluxes during ethacrynic acid-induced choleresis in three perfusion experiments. ■, Bile flow; ▲, biliary excretion of $^{24}Na^+$ (values are corrected for canalicular [25] and dead space of the cannula); ●, $^{24}Na^+$ in perfusate.

Bile/liver concentration ratios (Table 3) are consistent with earlier observations of Klaassen and Fitzgerald [16], who suggested an active transfer of ethacrynic acid metabolites into bile. Saturable canalicular transport of the ethacrynic acid glutathione adduct is illustrated in Fig. 4 (lower part). The maximal velocity of this pathway (0.15 μmoles. min^{-1}.g $liver^{-1}$) is considerably lower than the V_{max} of the uptake process. Canalicular excretion of the ethacrynic acid conjugate, therefore, is the rate-limiting step in transfer of ethacrynic acid from 'plasma' to bile.

Changes in Na^+ *fluxes accompanying hepatic ethacrynic acid transport.* Incubation (10 min) of liver slices in a medium containing 0.495 μmoles/ml ethacrynic acid raises intracellular sodium to 112.0 ± 3.9 (S.E.) per cent over controls (100.0 ± 2.7 per cent, N = 15 slices per group). Though small, the difference is statistically significant ($P<0.025$). At the same time, intracellular potassium fell to 93.2 ± 1.7 per cent of the control value (100.0 ± 1.9 per cent, $P<0.025$).

In a second line of experiments, the influence of ethacrynic acid on Na^+ efflux into perfusate and bile was studied in livers preloaded with $^{24}Na^+$. Figure 5 shows that infusion of ethacrynic acid causes a slower release of $^{24}Na^+$ into the perfusate which becomes more pronounced at the end of the infusion period. It is not possible to distinguish whether this alteration is due to direct inhibition of Na^+ efflux or is the consequence of decreasing intracellular specific activity following increased influx of 'cold' Na^+ from the perfusion medium. Significant changes in Na^+ efflux were also observed at the canalicular site (Fig. 5). During ethacrynic acid-induced choleresis, a pronounced stimulation of $^{24}Na^+$ extrusion into bile was observed.

DISCUSSION

The present study provides evidence that entry of the choleretic drug ethacrynic acid into liver cells involves, apart from diffusion, a saturable, energy-dependent transfer step whose maximal velocity displays partial sensitivity to extracellular Na^+. The apparent Michaelis–Menten constant K_m, reflecting the carrier affinity towards the transported substrate, remains virtually unchanged by replacement of Na^+ in the perfusion medium. As far as the kinetic con-

stants are concerned, ethacrynic acid transport follows the same pattern of Na^+-sensitive transport as described for other choleretics such as ouabain, cholic and taurocholic acid [9, 10, 19].*

The significance of the second, slow component of ethacrynic acid disappearance from perfusion medium is quite unclear. It might represent saturation of conjugation which could occur without any depletion of the glutathione pool, although the distribution experiments do not point in that direction.

The observed Na^+-sensitivity of sinusoidal ethacrynic acid transport stimulated the search for direct evidence for coupled Na^+ and ethacrynic acid transfer. Because direct measurements of Na^+ influx in the isolated liver are affected with a high degree of uncertainty [21], this precludes any detection of relatively small changes in this transmembrane Na^+ movement. However, measurement of $^{24}Na^+$ efflux, as described, reveals some changes in sinusoidal Na^+ fluxes consistent with increased inward diffusion of Na^+ during simultaneous translocation of ethacrynic acid. Also, the net increase of intracellular Na^+ during short-term incubation of liver slices with choleretic concentrations of ethacrynic acid could be the result of increased inward flow of Na^+ from the extracellular space and/or inhibition of outward Na^+ movements. Considering the fact that the canalicular membrane encompasses only a small fraction of the total liver cell surface [22], it is likely that the observed net increase in intracellular sodium implies mainly changes in Na^+ fluxes on the blood side of the liver. Co-transport of Na^+ and ethacrynic acid alone or in combination with inhibition of the sinusoidal (Na^+-K^+)-ATPase would lead to increased net flux of sodium into liver cells. The latter possibility cannot be disregarded as, within the concentration range employed, the drug effectively inhibits (Na^+-K^+)-ATPase [2]. This would also explain the observed decrease of intracellular K^+. On the other hand, a rearrangement of intracellular electrolytes (including K^+) as a consequence of enhanced Na^+ influx cannot be excluded [23].

Unequivocally, transport of the glutathione metabolite of ethacrynic acid into bile canaliculi is accompanied by increased biliary excretion of $^{24}Na^+$. This is undoubtedly of intracellular origin and could not have re-entered the bile canaliculi from the perfusate via a paracellular route, since under the given experimental conditions the specific activity of $^{24}Na^+$ in bile is considerably higher than in perfusate [11, 24]. Direct stimulatory interaction of the ethacrynic acid derivative with a canalicular sodium pump and/or increased intracellular Na^+ concentration would both lead to activation of Na^+ extrusion into bile canaliculi and could, therefore, be responsible for additional fluid (bile) production. This provides an explanation for the fraction of bile not secreted under the influence of the excreted ethacrynic acid metabolite.

* The failure to detect any major influence of Na^+ on ouabain uptake in isolated liver cells [20] might be due to substantial differences in experimental conditions.

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