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MECHANISTIC ASPECTS OF UPTAKE AND SINUSOIDAL EFFLUX OF DIBROMOSULFOPHTHALEIN IN THE ISOLATED PERFUSED RAT LIVER

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Abstract—Using the isolated perfused rat liver technique we examined whether the accumulation and sinusoidal efflux processes of the organic anion dibromosulfophthalein (DBSP) are dependent on the intracellular ATP content, chloride concentration in the perfusion medium as well as temperature of the medium and whether they are mediated by the same or by separate carrier mechanisms. The net sinusoidal efflux rate, being the resultant of sinusoidal efflux and re-uptake, was decreased more than 50% after lowering the medium temperature from 37 to 26° indicating that the efflux process is carrier-mediated. The uptake rate was decreased only 18% after lowering the medium temperature to 26°. Lowering of the hepatic ATP content for more than 80% clearly decreased the DBSP uptake rate but not the sinusoidal efflux rate. These observations indicate that these opposing transport steps probably are mediated by two separate carriers. Additional evidence for this hypothesis originated from the observation that sinusoidal efflux of DBSP was decreased about 30% whereas hepatic uptake of the substrate remained unaltered after replacing chloride in the perfusion medium with gluconate. In summary, we conclude that uptake and sinusoidal efflux of DBSP are mediated by two separate carrier systems that are influenced differently by ATP depletion, temperature lowering and presence of Cl^- gradients.

Uptake of organic anions by the liver is a powerful transport process since even very highly albumin bound compounds are efficiently removed from the general circulation [1, 2]. Transport across a membrane can in principle occur by passive permeation of the lipoid structure or can be carrier-mediated. Furthermore, carrier-mediated transport can be either passive or active. In the latter case transport is indirectly (via ion-gradients) or directly (via ATP) coupled to intracellular energization (secondary and primary active transport, respectively).

Many studies have addressed the nature of the hepatic uptake process of organic anions like tetrabromosulfophthalein (BSP†) and its dibromo analogue dibromosulfophthalein (DBSP). Accumulating evidence indicates that hepatic transport of these diagnostic dyes is carrier-mediated: uptake of BSP and DBSP in isolated hepatocytes was shown to be saturable [3, 4] and temperature sensitive [3, 5]. Uptake of both BSP and DBSP into hepatocytes is competitively inhibited by ICG [3, 4]. In addition, countertransport has been shown for BSP *in vivo* [6] and in isolated hepatocytes [7]. This latter phenomenon also reflects the bidirectional character of the membrane transport.

As with respect to the energization and ion-dependency of the uptake process there is little agreement in the current literature. Uptake of BSP was not substantially affected by metabolic inhibitors like KCN, 2,4-dinitrophenol and rotenon [4, 7, 8] whereas in another study [5] sodium azide and 2-deoxyglucose decreased both uptake and efflux of the dye in short-term cultured hepatocytes. For DBSP uptake, one study showed that dinitrophenol or KCN had no effect on uptake, whereas in the same study this transport step could be inhibited by using carbonylcyanid *m*-chlorphenylhydrazone (CCCP) or antymicin A [3]. Yamazaki *et al.* [8] showed that the ATP depleting agents rotenon, carbonylcyanide - *p* - trifluoromethoxyphenylhydrazone (which is related to CCCP) and sodium azide reduced the uptake of DBSP in isolated hepatocytes for 60% while surprisingly uptake of BSP was unaffected.

Studies in liver sinusoidal vesicles and short-term cultured hepatocytes suggested some relationship of BSP uptake to that of chloride by an as yet not clarified anion-exchange mechanism or a Cl^--modulating mechanism [5, 9, 10]. Both uptake and excretion of the anion were decreased after Cl^- substitution [5].

Following uptake into the hepatocytes organic compounds can be metabolized and excreted into the bile or back into the blood stream. The transport of some organic anions into the bile canaliculus is very likely carrier-mediated. This has been demonstrated in interaction studies *in vivo* and was

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† Abbreviations: BSP, tetrabromosulfophthalein; DBSP, dibromosulfophthalein; CCCP, carbonylcyanid *m*-chlorphenylhydrazone; BSA, bovine serum albumin; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

confirmed in studies using canalicular membrane vesicles [11–14]. The canalicular excretion of (D)BSP and dinitrophenyl-glutathione was shown to be ATP dependent [3, 5, 15, 16]. So far, only limited studies have addressed the nature of the sinusoidal efflux process for organic anions [16–19]. Carrier-mediated transport for glutathione, harmol-sulfate and dinitrophenyl-glutathione was inferred from studies by Ookhtens *et al.* [18], de Vries *et al.* [17] and Oude Elferink *et al.* [16]. Due to its relatively high hydrophilicity and the presence of at least two strongly acidic functional groups in the molecule, carrier-mediated sinusoidal efflux of DBSP is very likely.

The aim of the present study was to elucidate the nature of the hepatic sinusoidal efflux of the organic anion DBSP with respect to temperature dependency, energization of the overall sinusoidal efflux process and the dependency on chloride, and to compare it with the uptake process in order to find out whether uptake and efflux are mediated by the same or by separate carrier mechanisms.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing from 231 to 278 g were kept in a controlled environment with a 12-hr dark–light cycle. The animals were fasted overnight before surgery.

Chemicals

3,6-Dibromosulfophthalein (DBSP) was obtained from Societé d'Etudes et de Recherches Biologiques (Paris, France). BSA was from Organon Teknika (Boxtel, The Netherlands). Dextran T-70 (DT-70), mean molecular weight 70 kDa, was from Pharmacia (Uppsala, Sweden). All other chemicals were of analytical grade and obtained from commercial sources.

Isolated perfused rat liver

The surgical technique and design of the perfusion apparatus used in these studies was essentially the same as described previously* [20].

Temperature dependency. In the perfusion experiments which were used to study the temperature dependency of the net sinusoidal efflux we preloaded isolated perfused rat livers with DBSP. Livers were perfused single pass using perfusion medium containing no BSA but 2% (w/v) DT-70 in order to partly restore colloid-osmotic pressure. After a stabilization period of 30 min, livers were preloaded with 26.7 μmol DBSP per kg body weight by infusing the dye into the portal vein tubing as a solution of 0.797 mM in Krebs–Henseleit containing 10 mM HEPES, pH 7.4 at a rate of 0.94 mL/min. At the end of this period medium was rapidly changed to Krebs–Henseleit bicarbonate buffer having a temperature of 26° or 37° (control experiments) and containing 11.1 mM D(+)-glucose, 1.95% DT-70 (w/v) and 7.5 μM BSA. We measured the net sinusoidal efflux rate in perfusate at regular intervals during the 45 min efflux period. Experiments that were used to study the temperature dependency of the uptake process were performed as described for ATP dependency control experiments except that the temperature of the perfusion medium during DBSP infusion was 26°.

ATP dependency. Isolated perfused rat livers were used to examine the ATP dependency of both hepatic uptake and sinusoidal efflux of DBSP. *Hepatic uptake:* After stabilizing livers for 30 min with Krebs–Henseleit bicarbonate buffer containing 2% DT-70 (w/v) and no albumin, ATP was depleted by perfusing livers single pass for 15 min with medium containing 11.1 mM D(−)-fructose, 2% DT-70 (w/v) and 10 μM CCCP (an uncoupler of the oxidative phosphorylation) or 10 min with the same medium without CCCP. In the control experiments CCCP was omitted from the perfusion medium and fructose was replaced by D(+)-glucose. Subsequent to the depletion period we determined the uptake of DBSP by measuring the initial extraction of DBSP infused at a final medium concentration of 24.0 ± 1.50 μM (mean ± SD, N = 8). Perfusate sampled from 20 to 30 sec after initiation of the DBSP infusion was used to determine the initial extraction (which is a measure for uptake). In all experiments, including the control experiments, bile duct was ligated after stabilizing the livers because during ATP depletion bile flow strongly decreases. *Sinusoidal efflux:* The ATP dependency of the sinusoidal efflux was studied after preloading rat livers as described for the temperature dependency studies of the net sinusoidal efflux process. Subsequently, the bile duct was ligated and ATP was depleted as was done in the uptake experiments. We previously showed* that sinusoidal efflux can be studied best at high albumin concentration in the perfusion medium. Under this condition re-uptake of ligand excreted into the medium is relatively low due to very high protein binding. Therefore, we measured initial sinusoidal efflux rate for 10 sec in perfusate containing 600 μM BSA. The ATP content in liver was determined directly after perfusate sampling as described under analytical procedures.

Chloride dependency. Similarly as for ATP dependency, we used separate experimental settings in order to study the chloride dependency of both the hepatic uptake and sinusoidal efflux of DBSP. *Initial extraction:* Initial extraction was measured after a 30 min stabilization period and a subsequent 2 min chloride depletion period during which time livers were perfused with medium in which all chloride salts were replaced by the corresponding gluconate salts. Gluconate salts were used as it has been described previously that this substitution did not alter oxygen consumption, perfusion pressure or enzyme release whereas the reduction in bile flow and membrane potential was reversible [19]. Perfusate sampled from 20 to 30 sec after subsequent initiation of the DBSP infusion was used to determine the initial extraction. *Sinusoidal efflux:* Sinusoidal efflux was measured in perfusate containing 600 μM BSA after preloading livers with DBSP (as described for the ATP dependency studies) and subsequent chloride depletion as described above. In both

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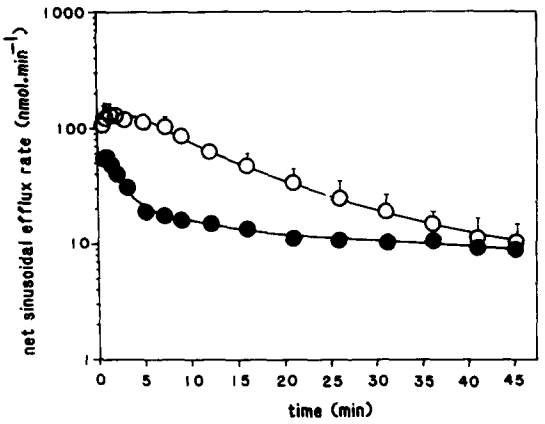

Fig. 1. Typical curve of the net sinusoidal efflux rate of DBSP after preloading and subsequent single pass perfusion with medium having a temperature of 26° (●). Bars in control experiments [37° (○)] denote SD of four experiments (only shown when exceeding size of symbols).

procedures chloride in perfusate was measured as described under analytical procedures.

In all perfusion experiments described so far, flow rate was established at 3.5 mL/min/g liver at a pressure of 7–15 cm H_2O. The pH of the perfusion medium was maintained between 7.35 and 7.48 by slightly altering the pCO_2. DBSP was determined as described below.

Analytical procedures

Perfusate samples were assayed for DBSP by adding 3.5% (v/v) 2 N NaOH, 126 mM Na_2EDTA and measuring absorption at 575 nm on a Philips PU 8700 spectrophotometer.

ATP was extracted from liver pieces excised and snap-frozen in liquid nitrogen directly after perfusate sampling. ATP was extracted using a solution of 7% perchloric acid and 40% ethanol (v/v) and was determined fluorimetrically as previously described [21]. Measurements of ATP were carried out with both internal and external standards.

Chloride concentration in perfusate samples was determined potentiometrically [22].

RESULTS

We measured the net sinusoidal efflux rate after preloading the liver with DBSP and subsequent lowering of the perfusion medium temperature. The net sinusoidal efflux rate is the resultant of actual sinusoidal efflux and subsequent re-uptake of ligand secreted into the sinusoids. Figure 1 shows a typical curve of the net sinusoidal efflux rate using perfusion medium having a temperature of 26°.

It is obvious that the net sinusoidal efflux rate is considerably lowered compared to control experiments in which the efflux medium temperature was 37°. All triple replicate experiments performed showed a pronounced decrease in net sinusoidal efflux rate after lowering the medium temperature, the inhibition ranging from 52 to 82%. In addition to a decrease in net sinusoidal efflux rate, the biliary excretion rate was decreased also (data not shown).

The observed difference in the net sinusoidal efflux rate between 26° and 37° is neither due to a difference in liver load of DBSP at the beginning of the efflux period as compared to the control experiments, nor is it due to a temperature induced decrease in medium flow as this was held constant. Uptake experiments performed at 26° showed that the initial extraction ratio of DBSP is only moderately decreased from 0.985 ± 0.014 at 37° (mean ± SD, N = 3) to 0.819 ± 0.024 (mean ± SD, N = 2).

We studied the energization of both the uptake and the efflux process of DBSP. After depleting the liver ATP content using CCCP (an uncoupler of the oxidative phosphorylation) the effect on both the initial extraction and the initial sinusoidal efflux rate of DBSP was determined. In both experimental settings the ATP content was decreased more than 80% (Table 1).

This severe lowering of the liver ATP content had a pronounced effect on DBSP uptake rate as the initial extraction was lowered 49.5% (Table 1). In contrast, the initial sinusoidal efflux rate of the substrate was not decreased and in fact was increased by 27% as compared to the control value (Table 1).

This ATP dependency of the uptake process was confirmed depleting cellular ATP with fructose. The initial extraction ratio was slightly but significantly ($P < 0.05$) decreased from 0.985 ± 0.014 for the control (mean ± SD, N = 3) to 0.909 ± 0.008 after depletion (mean ± SD, N = 2). The ATP content was decreased to 0.520 ± 0.010 μmol/g liver (mean ± SD, N = 2).

To further study possible differences in the uptake and efflux process we examined the influence of chloride replacement in the perfusion medium on both transport steps. All chloride salts in the perfusion medium were replaced by the corresponding gluconate salts. Cl^- concentration in the control perfusion medium was 126 ± 0.57 mM (mean ± SD, N = 3) and was decreased to 5.7 ± 0.58 mM (mean ± SD, N = 3) in the "chloride-free" perfusate. Despite a more than 22-fold reduction in chloride concentration no effect on the initial extraction of DBSP was observed. The initial sinusoidal efflux rate however, showed a dependency on chloride as it was significantly lowered to 69.5% of the control rate (Table 2).

We estimated the intracellular chloride concentration at the end of the Cl^- depletion period by collecting all perfusate during this period and measuring the total chloride concentration. In addition, we measured the chloride concentration in perfusate samples after starting the "chloride-free" perfusion period and just prior to measuring the initial extraction (at 2 min 20 sec). These data indicated that little, if any, chloride was still present intracellularly after the "chloride-free" perfusion.

DISCUSSION

In this study we examined some mechanistic aspects of basolateral transport of the organic anion dibromosulfophthalein (DBSP). We investigated the dependency of both hepatic uptake and sinusoidal

Table 1. ATP dependency of uptake and sinusoidal efflux of DBSP

	Uptake			Sinusoidal efflux		
	Initial extraction (fraction of dose)	(%)	ATP content (μmol/g liver)	Rate constant ($\times 10^{-3}$ min^{-1})	(%)	ATP content (μmol/g liver)
Control	0.985 ± 0.014	100	0.570 ± 0.051	71.6 ± 3.0	100	0.570 ± 0.019
ATP depleted (with CCCP)	0.497 ± 0.011*	50.5	0.041 ± 0.007*	90.9 ± 5.3*	127	0.115 ± 0.041*

Data are mean ± SD of three experiments. Values are expressed as absolute values or as percentage of control (%).
* Statistically different from control ($P < 0.05$).

Table 2. Chloride dependency of uptake and sinusoidal efflux of DBSP

	Uptake		Sinusoidal efflux	
	Initial extraction (fraction of dose)	(% of control)	Rate constant ($\times 10^{-3}$ min^{-1})	(% of control)
Control	0.985 ± 0.014 (3)	100	71.70 ± 3.01 (3)	100
Cl^- depleted	0.991 ± 0.001 (4)	101	49.87 ± 6.69* (4)	69.5

Data are mean ± SD of the number in parentheses.
* Statistically different from control ($P < 0.05$).
Rate constant is defined as fraction of initial liver load excreted per minute.

efflux of DBSP on intracellular ATP content, chloride concentration in the perfusion medium as well as medium temperature in the isolated perfused rat liver and examined whether uptake and efflux are mediated by the same or by separate carrier mechanisms.

Temperature dependency

Net sinusoidal efflux rate as measured in perfusate is, as was shown before*, the resultant of sinusoidal efflux and subsequent partial re-uptake of ligand. Our results show that this net sinusoidal efflux is strongly temperature dependent. Various authors have shown that hepatic uptake of DBSP [3, 17] and that of the structurally related compound BSP [5, 7, 23–25] is very likely carrier-mediated. Blom *et al.* [3] showed a temperature dependency of initial uptake of DBSP in isolated rat hepatocytes.

The fact that the *net* sinusoidal efflux is largely lowered indicates that the actual sinusoidal efflux process is strongly temperature dependent. Furthermore, the present experiments show that DBSP uptake by the perfused rat liver is clearly temperature dependent confirming the observations in isolated hepatocytes [3]. This decrease is probably not due to a decreased ATP content as it was shown in isolated hepatocytes† that after lowering the temperature of the incubation medium to 26° ATP content is not reduced.

* Nijssen HMJ, Pijning T, Meijer DKF and Groothuis GMM, Influence of albumin on the net sinusoidal efflux of the organic anion dibromosulfophthalein from rat liver. *Hepatology*, submitted.

† Steen H, personal communication.

ATP dependency

We determined the ATP dependency of both uptake and sinusoidal efflux and found that only the initial extraction of DBSP and not the sinusoidal efflux rate is reduced after ATP depletion (Table 1). Additional experiments clearly showed that the initial extraction is decreased too after depleting cellular ATP with fructose, although to a lesser extent than after depleting with CCCP. This is in line with observations showing the depletion by fructose only is not as high as it is using fructose combined with CCCP [26]. For uptake of BSP [5] and DBSP [3, 8] in isolated hepatocytes using several metabolic inhibitors, ATP dependency was observed. However, other studies showed an ATP independency for uptake of BSP [4, 7].

Data on the ATP dependency of cellular efflux of (D)BSP published so far cannot discriminate between sinusoidal efflux and biliary excretion as these experiments were performed using isolated or cultured hepatocytes in which excretion via both processes occurs into the same medium. Therefore, until now, no data are available with regard to the influence of ATP depletion on the sinusoidal efflux process of organic anions *per se*. In the perfusion set-up we are able to discriminate kinetically between the processes of uptake, sinusoidal efflux and biliary excretion. The present results indicate that sinusoidal efflux of DBSP at least under the conditions tested, is not dependent on intracellular ATP. In the light of the above-mentioned effect of ATP depletion on the hepatic uptake process it is likely that the moderate increase found in the net sinusoidal efflux rate upon ATP depletion is due to a reduction in

re-uptake. As described before,* perfusing preloaded livers with medium containing 4% BSA is a tool for studying the sinusoidal efflux process *per se* with minor interference by re-uptake of ligand effluxed into the sinusoids. In the presence of 4% BSA in the perfusion medium the initial extraction by uptake of DBSP is approximately 10%. This means that under the present conditions a modest part of the ligand effluxing into the sinusoid undergoes re-uptake during liver passage. The lowering of re-uptake by ATP depletion therefore at least partly explains the observed increase in the net sinusoidal efflux rate.

In our experiments ATP was depleted using CCCP, a compound that uncouples oxidative phosphorylation in mitochondria through dissipation of the proton gradient across the inner mitochondrial membrane. It is in principle possible that due to this ATP depletion with CCCP the inside to outside proton gradient across the plasma membrane is also dissipated and/or that the membrane potential is influenced. Steen *et al.* [27] showed that the proton gradient in isolated hepatocytes was not altered after addition of CCCP. It was demonstrated by microelectrode techniques that 15 min after addition of 10 μM CCCP the plasma membrane potential is not altered in isolated hepatocytes.† Uptake of DBSP in isolated hepatocytes was earlier shown to be independent of membrane potential [3].

Our experiments show that hepatic uptake rather than sinusoidal efflux of DBSP is ATP dependent. We therefore conclude that uptake and sinusoidal efflux of the compound is probably mediated by two separate carriers.

Chloride dependency

We observed a significant reduction of the initial sinusoidal efflux rate after depleting the perfusion medium of chloride (Table 2). Wolkoff *et al.* [5] have previously discussed the existence of a Cl^-/organic anion exchange mechanism for basolateral BSP and bilirubin transport. More recently [10] the influence of chloride was described in terms of modulating the affinity of organic anions for the carrier without a coupling of Cl^- transport or Cl^- gradients. Our observation that sinusoidal efflux rather than initial extraction is reduced after depletion of chloride in the medium is consistent with the existence of a Cl^-/organic anion exchange mechanism in the case of the efflux process or alternatively could be explained by a modulating influence of Cl^- at the inner side of the membrane since perfusion with chloride-free medium also reduced intracellular chloride concentration.

In contrast to the sinusoidal efflux process, the initial extraction of DBSP was not reduced after depletion of chloride in the medium indicating that the uptake process for DBSP is not modulated by the inorganic ion. This may add to other observations [8] that uptake mechanisms of BSP and DBSP are dissimilar. As mentioned in the results, the intracellular chloride concentration after perfusion with Cl^- substituted medium is expected to be very low due to passive leakage of the inorganic anion across the membrane. Active transport mechanisms of Cl^- across the plasma membrane have not been described and the presence of an inwardly directed Cl^- gradient across the basolateral plasma membrane under basal conditions is due to the negative (inside to outside) membrane potential [28].

On the basis of the differential effect of Cl^- substitution we conclude that uptake of DBSP occurs by a mechanism dissimilar to that for the sinusoidal efflux process and also that Cl^- plays no major role in modulating the hepatic uptake of DBSP in contrast to that of BSP [5, 10]. However, our results do strengthen the conclusion that hepatic uptake and sinusoidal efflux of DBSP are mediated by two separate carriers. Additional evidence for this hypothesis originates from preliminary experiments showing that antibodies directed against bili-translocase, the putative carrier protein for organic anions like BSP clearly inhibited sinusoidal efflux of DBSP in isolated perfused rat livers whereas pharmacokinetic analysis indicated that uptake of DBSP was not decreased.

In conclusion, we observed a marked temperature dependency of the sinusoidal efflux process while the liver to plasma transport of DBSP is ATP independent but partially chloride dependent. In contrast, hepatic uptake of DBSP was shown to be at least partly ATP dependent and independent on a chloride gradient. We conclude that uptake and sinusoidal efflux of DBSP both occur via membrane carrier translocating mechanisms but that these transport steps are mediated by two separate carrier systems.

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REFERENCES

1. Meijer DKF, Transport and metabolism in the hepatobiliary system. In: *Handbook of Physiology. Vol III. The Gastrointestinal System* (Eds. Schultz SG, Forte JG and Rauner BB), pp 717–758. The American Physiological Society, Bethesda, 1989.
2. Berk PD and Stremmel W, Hepatocellular uptake of organic anions. In: *Progress in Liver Diseases* (Eds. Popper H and Schaffner F), Vol. VIII, pp. 125–144. Grune and Stratton, New York, 1986.
3. Blom A, Keulemans K and Meijer DKF, Transport of DBSP by isolated rat hepatocytes. *Biochem Pharmacol* **30**: 1809–1816, 1981.
4. Schwenk M, Burr R, Schwarz L and Pfaff E, Uptake of bromosulfophthalein by isolated liver cells. *Eur J Biochem* **64**: 189–197, 1976.
5. Wolkoff AW, Samuelson AC, Johansen KL, Nakata R, Withers DM and Sosiak A, Influence of Cl^- on organic anion transport in short-term cultured rat hepatocytes and isolated perfused rat liver. *J Clin Invest* **79**: 1259–1268, 1987.

* Nijssen HMJ, Pigning T, Meijer DKF and Groothuis GMM, Influence of albumin on the net sinusoidal efflux of the organic anion dibromosulfophthalein from rat liver. *Hepatology*, submitted.

† Steen H, personal communication.

6. Scharschmidt BF, Waggoner JG and Berk PD, Hepatic organic anion uptake in the rat. *J Clin Invest* **56**: 1280–1292, 1975.
7. Stremmel W and Berk PD, Hepatocellular uptake of sulfobromophthalein and bilirubin is selectively inhibited by an antibody to the liver plasma membrane sulfobromophthalein–bilirubin binding protein. *J Clin Invest* **78**: 822–826, 1986.
8. Yamazaki M, Suzuki H, Sugiyama Y, Iga T and Hanano M, Uptake of organic anions by isolated rat hepatocytes: a classification in terms of ATP-dependency. In: *The Third International Congress on Mathematical Modelling of Liver Excretory Processes*, pp. 322–327, 1990.
9. Potter BJ, Blades BF, Shepard MD, Thung SM and Berk PD, The kinetics of sulfobromophthalein uptake by rat liver sinusoidal vesicles. *Biochim Biophys Acta* **898**: 159–171, 1987.
10. Wolkoff AW, Studies of the mechanism of organic anion transport in isolated perfused rat liver and short-term rat hepatocytes. In: *The Third International Congress on Mathematical Modelling of Liver Excretory Processes*, pp. 49–54, 1990.
11. Berk PD, Potter BJ and Stremmel W, Role of plasma membrane ligand-binding in the hepatocellular uptake of albumin-bound organic anions. *Hepatology* **7**: 165–176, 1987.
12. Inoue M, Kinne R, Tran T and Arias IM, Taurocholate transport by rat liver canalicular membrane vesicles. *J Clin Invest* **73**: 659–663, 1984.
13. Inoue M, Kinne R, Tran T, Diempica L and Arias IM, Rat liver canalicular membrane vesicles. Isolation and topological characterization. *J Biol Chem* **258**: 593–598, 1983.
14. Thalhammer T, Hansel G and Graf J, Analysis of hepatic uptake and biliary excretion of an anionic xenobiotic utilizing isolated plasma membrane vesicles. In: *Pharmacochemistry Library VIII* (Ed. Tichy M). Elsevier, Amsterdam, 1985 (Proc Symp QSAR Toxicol Xenobiochem).
15. Kobayashi K, Sogane Y, Hayashi K, Nicotera P and Orrenius S, ATP stimulates the uptake of *S*-dinitrophenylglutathione by rat liver plasma membrane vesicles. *FEBS Lett* **240**: 55–58, 1988.
16. Oude Elferink RPJ, Ottenhof R, Liefting WGM, Schoemaker B, Groen AK and Jansen PLM, ATP-dependent efflux of GSSG and GS-conjugate from isolated rat hepatocytes. *Am J Physiol* **258**: G699–G706, 1990.
17. De Vries MH, Groothuis GMM, Mulder GJ, Nguyen H and Meijer DKF, Secretion of the organic anion harmol sulfate from the liver into blood. Evidence for a carrier mediated mechanism. *Biochem Pharmacol* **43**: 2129–2135, 1985.
18. Ookhtens M, Hobdy K, Corvasce MC, Aw TY and Kaplowitz N, Sinusoidal efflux of glutathione in the perfused rat liver. Evidence for a carrier-mediated process. *J Clin Invest* **75**: 258–265, 1985.
19. Wright TL, Fitz JG and Boyer TD, Hepatic efflux of glutathione by the perfused rat liver: role of membrane potential difference. *Am J Physiol* **255**: G547–G555, 1988.
20. Meijer DKF, Keulemans K and Mulder GJ, Isolated perfused rat liver technique. *Methods Enzymol* **77**: 81–94, 1981.
21. Williamson JR and Corkey BE, Assays of intermediates of the citric acid cycle and related compounds by fluorimetric enzyme methods. *Methods Enzymol* **13**: 434–513, 1969.
22. Cotlove E, Trantham HV and Bowman RL, An instrument and method for automatic, rapid, accurate, and sensitive titration of chloride in biologic samples. *J Lab Clin Med* **51**: 461–468, 1958.
23. Goresky CA, Initial distribution and rate of uptake of sulfobromophthalein in the liver. *Am J Physiol* **207**: 13–26, 1964.
24. Goresky CA, The hepatic uptake and excretion of sulfobromophthalein and bilirubin. *Can Med Ass J* **92**: 851–857, 1965 .
25. Sugiyama Y, Kimura S, Lin JH, Izukura M, Awazu S and Hanano M, Effects of organic anions on the uptake of 1-anilino-8-naphthalene-sulfonate by isolated liver cells. *J Pharmac Sci* **72**: 871–876, 1983.
26. Nishi T, Kido Y, Furuya E, Tagawa K and Mori T, The effect of fructose on the cellular content of adenine nucleotides in the perfused rat liver. *Jpn J Surg* **19**: 351–357, 1989.
27. Steen H, Oosting R and Meijer DKF, Mechanisms for uptake of cationic drugs in the liver. A study with tributylmethylammonium (TBuMA) concerning substrate specificity and potential driving forces. *J Pharmacol Exp Ther*, in press.
28. Van Dyke RW, Lake JR and Scharschmidt BF, Cellular mechanisms of hepatic fluid and electrolyte transport. In: *Handbook of Physiology. Vol. III. The Gastrointestinal System* (Ed. Ranner BB), pp. 597–619. The American Physiological Society, Bethesda, 1989.