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ACINAR HETEROGENEITY IN HEPATIC TRANSPORT OF DIBROMOSULFOPHTHALEIN AND OUABAIN STUDIED BY AUTORADIOGRAPHY, NORMAL AND RETROGRADE PERFUSIONS AND COMPUTER SIMULATION

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Abstract—This study is aimed to investigate the relative involvement of periportal (zone 1) and perivenous (zone 3) hepatocytes in the uptake and biliary excretion of the organic anion dibromosulfophthalein (DBSP) and the uncharged cardiac-glycoside ouabain. The localization in the acinus of [^{35}S]BSP (sulfobromophthalein, the tetra-bromo-analogue of DBSP) and [^{3}H]ouabain administered to livers perfused with normal and retrograde flow, was detected by autoradiography. The plasma disappearance and biliary excretion rates of DBSP and [^{3}H]ouabain were determined in normal and retrograde perfusions. In addition, computer simulations were performed to predict the effect of reversal of the perfusate flow on the plasma disappearance and biliary excretion rate curves and on the concentration of label in zones 1 and 3.

Autoradiography showed that 2 and 10 min after injection of [^{35}S]BSP to normally and retrogradely perfused livers, the label was uniformly distributed in the liver acinus. The same results were found 30 sec and 10 min after injection of [^{3}H]ouabain to normally and retrogradely perfused livers. The plasma disappearance and biliary excretion rate of DBSP were slightly faster in retrograde perfusions compared to normal perfusions both with and without a basal bile salt infusion of 15 μmole/hr. This could not be explained by an acinar heterogeneity with respect to any of the DBSP transport steps (plasma to liver, liver to plasma, liver to bile) as was shown by computer simulations. The plasma disappearance and biliary excretion rate of ouabain were similar in normal and retrograde perfusions.

It is concluded that periportal and perivenous hepatocytes are equally involved in the uptake of (D)BSP and ouabain from the medium. However, due to the particular distribution patterns no conclusions can be drawn from normal and retrograde perfusions about the relative involvement of these cells in biliary excretion, as was shown by computer simulation. The unaffected kinetic behaviour of the retrogradely perfused livers indicated that no liver damage occurs during retrograde perfusion with respect to transport function.

The smallest microcirculatory unit of the liver, the acinus [1] is commonly divided into different zones of cells according to their localization with respect to the incoming blood. The cells around the afferent terminal portal venules, the periportal or zone 1 cells, differ from those around the efferent hepatic venules, the perivenous or zone 3 cells, in microenvironment, ultrastructure and enzymatic composition. Evidence for a functional heterogeneity between zone 1 and zone 3 cells in metabolic functions is accumulating (see [2–4] for reviews).

In recent years, increasing evidence has been obtained for a heterogeneous involvement of periportal and perivenous cells in the liver transport of compounds from blood to bile. Fluorescent microscopy [5], microdensitometry [6] and autoradiography [7, 8] were used to demonstrate the existence of concentration gradients in the cells of the acinus. However, most of these studies could not elucidate whether these concentration gradients occurred as a consequence of intrinsic differences between these cells in the acinus with respect to transport or conjugation capacities, or whether they were due to concentration gradients in the sinusoids. Attempts were made to separate isolated hepatocytes into fractions enriched in periportal and perivenous cells to determine the transport functions of these cells separately [9]. However, although the obtained cell fractions appeared to differ slightly in the uptake rate of ouabain and taurocholate, no satisfactory evidence for the acinar origin of these subpopulations was obtained. In other studies zone selective toxins were used to investigate the acinar heterogeneity in bile salt [10–12] and DBSP (dibromosulfophthalein) transport [10].

Recently we described a method to study the transport capacities of zone 1 and zone 3 cells, in which we combined the autoradiographic localization of injected [^{3}H]taurocholate in the acinus with the kinetic analysis of the uptake and biliary excretion rate in isolated livers perfused with normal and

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retrograde direction of flow [13]. In this study we employed this method to investigate the acinar localization of the uptake and biliary excretion of the organic anion DBSP and the uncharged cardiac-glycoside ouabain. Both compounds are not metabolized in the rat and are efficiently excreted into the bile [14–17]. Because no radioactively labelled DBSP is available, [^{35}S]BSP (the tetrabromo-analogue of DBSP, which undergoes conjugation with glutathione in the liver), was used in the audoradiographic experiments. A computer simulation program was used to predict if changes could be expected in the plasma disappearance and biliary excretion rate curves upon reversal of the direction of the perfusate flow if zones 1 and 3 differ in either plasma to liver, liver to plasma or liver to bile transport rates. In addition, the theoretical ratio of the concentration of the substrate in zones 1 and 3 at various times after injection were calculated to predict if these differences in transport rate will result in detectable differences in grain densities on autoradiographs.

MATERIALS AND METHODS

Rats

Male Wistar rats, weighing 280–300 g, which had free access to food and water were used.

Materials

Dibromosulfophthalein (DBSP) was obtained from Société d'Etudes et de Recherche Biologique, Paris, France; [^{35}S]bromosulfophthalein ([^{35}S]BSP, 6.4 mCi/mmole) from Amersham; ouabain from Merck, Darmstadt, West Germany; [^{3}H]ouabain (19.3 Ci/mmole) from New England Nuclear Corp., Boston, MA, U.S.A.; bovine serum albumin from Poviet, Oss, Holland; indocyanine green (ICG) from Hynson, Westcott and Dunning Inc., Baltimore, U.S.A.; taurocholate from Fluka, AG, Buchs SG, Switzerland; Picofluor from Packard Instruments Comp. Inc., Downers Grove, IL, U.S.A. All other chemicals were from Merck, Darmstadt, West Germany.

Normal and retrograde perfusion

Rat livers were perfused in a recirculating perfusion set-up with normal or retrograde direction of flow as described previously [13]. The perfusion medium consisted of Krebs–bicarbonate buffer with 0.5% albumin and was constantly gassed with 95% O_2/5% CO_2. The flow was established at 40 ml/min at a constant perfusion pressure of 13 cm H_2O. The viability of the livers was checked as described previously [13].

For the determination of the plasma* disappearance and biliary excretion rate, 1850 nmole of DBSP or 26 nmole of [^{3}H]ouabain was administered to 100 ml of perfusion medium 30 min after starting the perfusion. In all the experiments with [^{3}H]ouabain and in one series of experiments with DBSP, a constant infusion of sodium taurocholate (15 μmole/hr) was started 30 min before the addition of DBSP or ouabain, to compensate for the loss of bile salts. Medium samples were taken at different time intervals, and bile was collected in 2 or 5 min fractions. In the ouabain experiments the initial extraction of ouabain by the liver was measured by taking medium samples at the inflow and outflow of the liver simultaneously at 1 min after injection. At the end of the experiments the livers were weighed. Small parts of the livers were used for histological staining and the remainder was homogenized.

For the autoradiography experiments 1 nmole of [^{3}H]ouabain (20 μCi) was injected into the inflow cannula in 5 sec, yielding an initial concentration of 0.26 nmole/ml. Pieces of liver were frozen 30 sec and 10 min after injection. For the [^{35}S]BSP autoradiographs 1850 nmole of [^{35}S]BSP (11.8 μCi) was injected into the 100 ml of perfusion medium, and pieces of liver were frozen at 2 min and 10 min after injection. No bile salt infusion was given in the [^{35}S]BSP experiments.

Chemical analysis

DBSP was measured by diluting plasma and bile samples with 0.1 N NaOH and by measuring the absorption at 575 nm. Radioactivity was measured after dissolving samples of plasma, bile and liver homogenates in 5–10 ml of Picofluor, using an Isocap liquid scintillation counter.

Autoradiography

The autoradiography procedure was performed as described in detail [13]. In this procedure the liver was kept frozen until development of the autoradiograph. In the dark room equipped with a red light 8 μm sections were cut and rapidly picked up on frozen slides coated with Ilford G5 emulsion in the cryostat at −20°. No frost mark was observed on the knife, indicating that section thawing did not occur. The sections were exposed in the dark at −90° for 3–6 weeks. After thawing the slides were developed, fixed and stained with hematoxylin and eosin.

One minor modification was introduced: to obtain better tissue preservation and to avoid loss of sections during the photographic fixation, tissue fixation (10 min at room temperature in 4% formaldehyde, 5.4% macrodex, 1% $CaCl_2$ in 0.9% NaCl) was carried out in the dark room after development and before the photographic fixation.

Pharmacokinetic analysis of the data

Plasma disappearance and biliary excretion rate curves were fitted with an iterative least-square regression computer program, yielding the best fit of the plasma disappearance and biliary excretion rate data. For DBSP, a two-compartmental model with elimination from the peripheral compartment yielded the best explanation for the combined plasma disappearance and biliary excretion rate patterns and the rate constants for transport from plasma to liver (k_{12}), from liver to plasma (k_{21}) and from liver to bile (k_{23}) were accordingly calculated. The total plasma clearance (Cl) was calculated from the dose and the area under the plasma disappearance vs time curve. The initial extraction ratio (E) was calculated using the formula $k_{12}V_1/Fl$, where V_1 is the volume of the

* The term plasma is also used to denote the perfusion medium.

central compartment and *Fl* is the medium flow through the liver.

For ouabain no satisfying fit of the combined plasma disappearance and biliary excretion rate data could be obtained, since the apparent terminal half-life ($t_{\frac{1}{2}}$) values of the plasma decay and biliary excretion rate curves within the experimental period were significantly different, as was also found by Blom *et al.* [14]. Therefore, only the $t_{\frac{1}{2}}$ values of the plasma curves were determined by fitting of the plasma disappearance curve, but no model parameters were calculated. The extraction ratio (E) was calculated from the amount of radioactivity present in the medium 1 min after injection, sampled before (C_{in}) and after (C_{out}) the liver using the formula $E = C_{in} - C_{out}/C_{in}$. The Cl of ouabain was calculated from the dose and the area under the plasma disappearance vs time curve.

All values are expressed as mean ± S.E. of the mean and statistical evaluation was performed using Student's t-test.

Computer simulations

A computer simulation program was used to investigate theoretically if and how the plasma disappearance and biliary excretion rate curves would change due to reversal of the blood flow through the liver. In addition this program was used to predict the ratio of the concentration of the substrate in the two zones at the timepoints when liver samples were taken for autoradiography.

With this computer program (developed in our laboratory by Dr. H. Hoving, unpublished data) the liver can be simulated by two compartments in series, as a model for the acinar zonation (zones 1 and 3), which is depicted in Fig. 1. The concentration in each of the compartments A–F was calculated at various times after a bolus injection of the test compound by solving the differential equations that describe these concentrations in course of time via standard matrix methods. A liver with heterogeneous localization of DBSP transport in the acinus was stimulated by varying the rate constants k_1 relative to k_3, k_2 relative to k_4 and k_5 relative to k_6. Reversal of the flow was stimulated by interchanging the values of k_1 and k_3 etc. The following simulations were performed:

(A) $k_1 = 3\ k_3$; $k_1 = 1/3\ k_3$; $k_1 = 9\ k_3$; $k_1 = 1/9\ k_3$; $k_1 = 99\ k_3$; $k_1 = 1/99\ k_3$ — $k_1 + k_3$ = experimentally found clearance for uptake. $k_2 = k_4$, $k_2 + k_4$ = experimentally found clearance for liver to plasma transport. $k_5 = k_6$, $k_5 + k_6$ = experimentally found clearance for liver to bile transport.

(B) k_2 and k_4 were varied accordingly, with $k_1 = k_3$ and $k_5 = k_6$.

(C) k_5 and k_6 were varied accordingly, with $k_1 = k_3$ and $k_2 = k_4$.

The concentrations in all compartments A–F were plotted vs time.

RESULTS

DBSP experiments

Perfusion. In Fig. 2 the plasma disappearance and biliary excretion rate of DBSP after administration of a single dose of 1850 nmole to isolated perfused livers with normal and retrograde direction of flow are given. The rate constants, calculated according to an open two-compartmental model with elimination from the peripheral compartment, the total plasma clearance (Cl), extraction (E), liver weight and bile flow are given in Table 1. These data indicate that slightly higher rate constants were found when livers were perfused retrogradely compared to the normal direction of flow. The same trends are observed when 1850 nmole DBSP was given during infusion of 15 μmole/hr taurocholate (Fig. 3, Table 1). In both normal and retrograde perfusions, bile flow and k_{23} are significantly increased during bile salt infusion.

Autoradiography. Two minutes after injection of [^{35}S]BSP to normally perfused livers, the label appeared homogeneously distributed in the liver acinus, although sometimes a slightly higher number of grains was seen in zone 3. No labelling of the bile ducts was detected at this time (Fig. 4). At 10 min after injection, a similar acinar distribution of grains was seen and in addition label was detected in the bile ducts (Fig. 5). The same localization was found for retrogradely perfused livers.

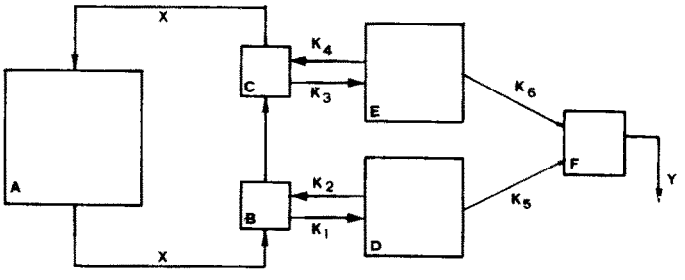

Fig. 1. Model used for the computer calculations in which the liver is simulated as two compartments in series. A is the plasma compartment; B and C are the plasma compartments in the liver; D and E are the two liver compartments, 'zone 1' and 'zone 3', respectively; F is the bile compartment; X is the plasma flow through the liver; Y is the bile flow; k_1–k_6 are the rate constants for the transport of DBSP.

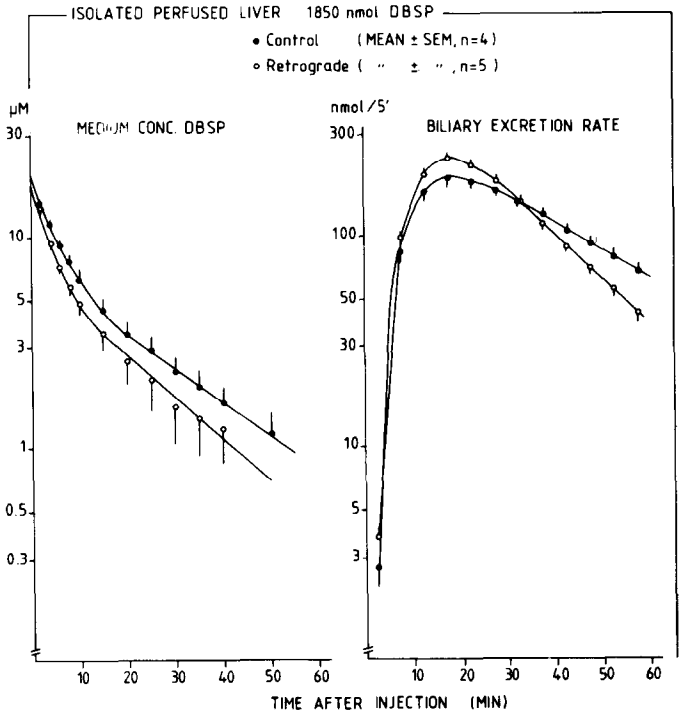

Fig. 2. Medium disappearance (left panel) and biliary excretion rate (right panel) of 1850 nmole DBSP administered to isolated perfused livers with normal (●) and retrograde (○) direction of perfusate flow.

Ouabain experiments

Perfusions. The plasma disappearance and biliary excretion rate curves obtained after administration of 26 nmole of [^{3}H]ouabain to normally and retrogradely perfused livers are given in Fig. 6. The curves were fitted according to a bi-exponential equation. The $t_{\frac{1}{2}}$ values of the plasma curves, the total plasma clearance (*Cl*), the extraction (*E*), bile flow and liver weight are given in Table 2. Except for the higher *E* in the retrograde perfusions, no significant differences were found in any of the parameters comparing the two directions of flow. It is evident from Fig. 5 that no differences in biliary excretion curves for ouabain are present.

Autoradiography. Autoradiographs of livers 30 sec after injection of [^{3}H]ouabain revealed a homogeneous labelling in the acinus both during normal and retrograde direction of flow, although in the latter sometimes a higher labelling of zone 3 was observed (Fig. 7). Bile duct labelling was evident 10 min after injection (Fig. 8), as well as homogeneous labelling of the acinus.

Simulations

In the computer simulations we varied the relative contribution of the two zones with respect to either the uptake process or one of the transport processes out of the cells, but keeping the sum of these pro-

Table 1. Hepatic transport of 1850 nmole DBSP in normal and retrograde perfusions

	No bile salt infusion		With taurocholate infusion	
	Normal (n = 4)	Retrograde (n = 5)	Normal (n = 6)	Retrograde (n = 4)
k_{12} (min^{-1})	0.138 ± 0.012	0.235 ± 0.019*	0.156 ± 0.020	0.183 ± 0.016
k_{21} (min^{-1})	0.034 ± 0.004	0.061 ± 0.017	0.032 ± 0.004	0.046 ± 0.014
k_{23} (min^{-1})	0.041 ± 0.006†	0.062 ± 0.004*†	0.069 ± 0.004	0.095 ± 0.009*
Cl (ml/min)	7.2 ± 0.8	11.5 ± 1.8	9.7 ± 0.7	12.4 ± 1.1
E (%)	34 ± 3	52 ± 2*	36 ± 3	47 ± 5
Bile flow (μl/min)	8.4 ± 0.5	10.9 ± 0.9	11.8 ± 0.8	13.3 ± 1.5
Liver weight (g)	9.4 ± 0.4	11.8 ± 0.6*†	10.5 ± 0.4	10.1 ± 0.4

The plasma disappearance and biliary excretion rate of 1850 nmole DBSP were measured in normal and retrograde perfusions with and without taurocholate infusion (15 μmole/hr). The rate constants for transport from plasma to liver (k_{12}), from liver to plasma (k_{21}) and from liver to bile (k_{23}) were calculated according to a two-compartmental model with elimination from the peripheral compartment. The total plasma clearance (*Cl*) was calculated from the dose and the area under the plasma disappearance vs time curve. The extraction (*E*) was calculated using the formula $k_{12}V_1/Fl$ where V_1 is the volume of the first compartment and *Fl* the flow through the liver. All values are given as mean ± S.E.

* Significantly different from normal perfusion, $P < 0.05$.

† Significantly different from experiments with taurocholate infusion, $P < 0.05$.

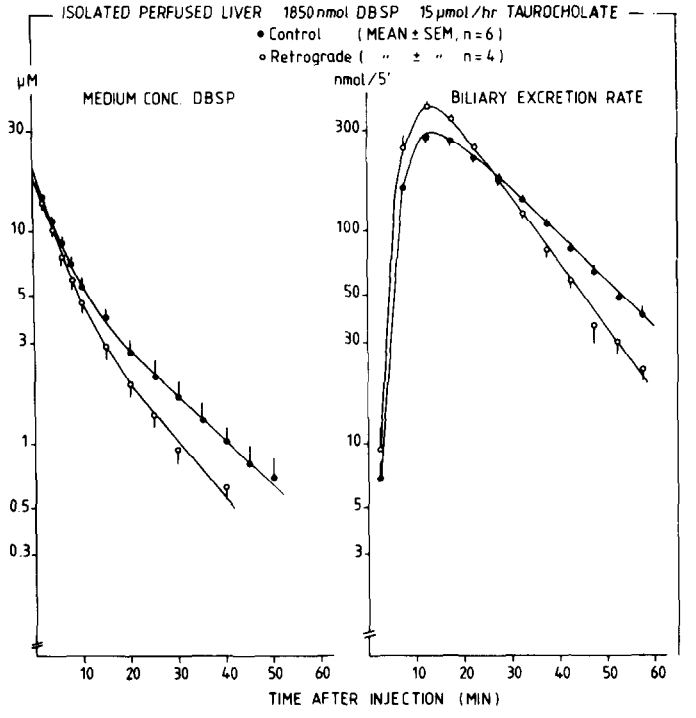

Fig. 3. Medium disappearance (left panel) and biliary excretion rate (right panel) of 1850 nmole DBSP administered to isolated perfused livers with normal (●) and retrograde (○) direction of perfusate flow, during infusion of 15 μmole/hr taurocholate.

cesses for the total liver constant. It appeared that in none of the simulated cases the profile of the plasma disappearance curve changed upon reversal of the flow. In addition, when the plasma to liver or liver to plasma rate constants in the various zones were varied, also no changes were observed in the biliary excretion rate curves due to reversal of the flow. However, when the clearance constants for

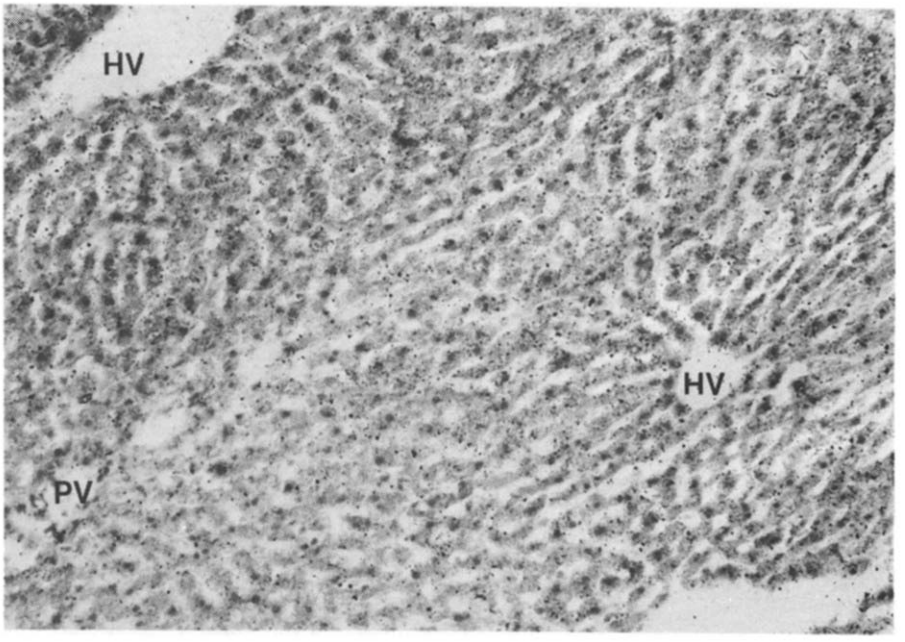

Fig. 4. Light microscopic autoradiograph of rat liver 2 min after injection of 1850 nmole [^{35}S]BSP into the medium reservoir of a perfused liver with normal direction of flow. The grains are homogeneously distributed in the acinus. The same distribution was observed in retrogradely perfused livers. PV, portal venule; HV, terminal hepatic venule (144×).

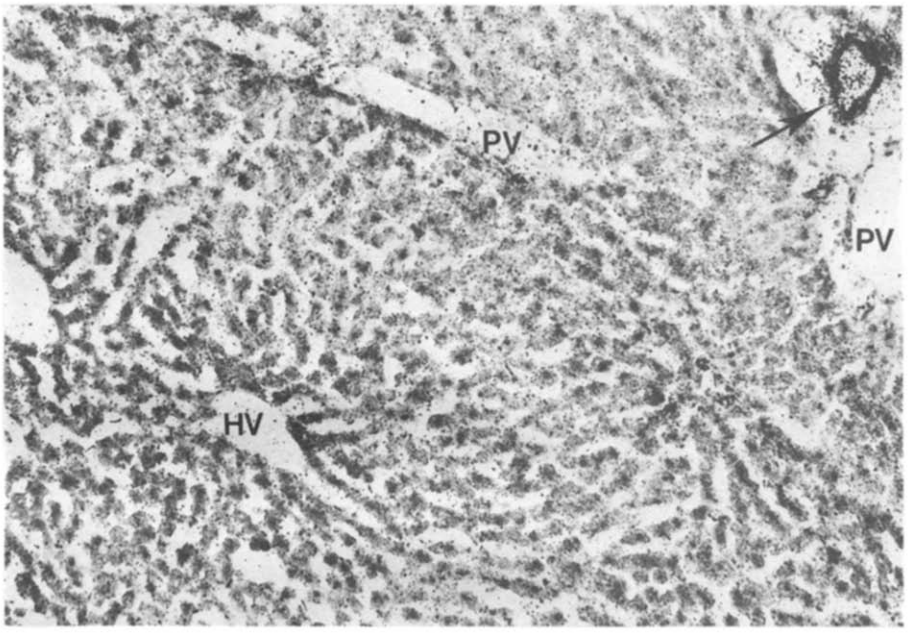

Fig. 5. Ten minutes after injection of 1850 nmole [^{35}S]BSP to a liver perfused with normal direction of flow. Grains are homogeneously distributed in the acinus, and bile ducts are labelled (arrow). The same distribution pattern was found 10 min after injection to retrogradely perfused livers. PV, portal venule; HV, terminal hepatic venule (144×).

biliary excretion were varied in the two liver compartments (while $k_1 = k_3$ and $k_2 = k_4$), the biliary excretion rate curves change upon the simulated reversal of the flow, but the differences were small. Slight differences were detectable, for instance when $k_5 = 99\ k_6$ was compared with $k_5 = 1/99\ k_6$: the top of the biliary excretion rate curves differed only 14% (Fig. 9). The slope of the descending phase was

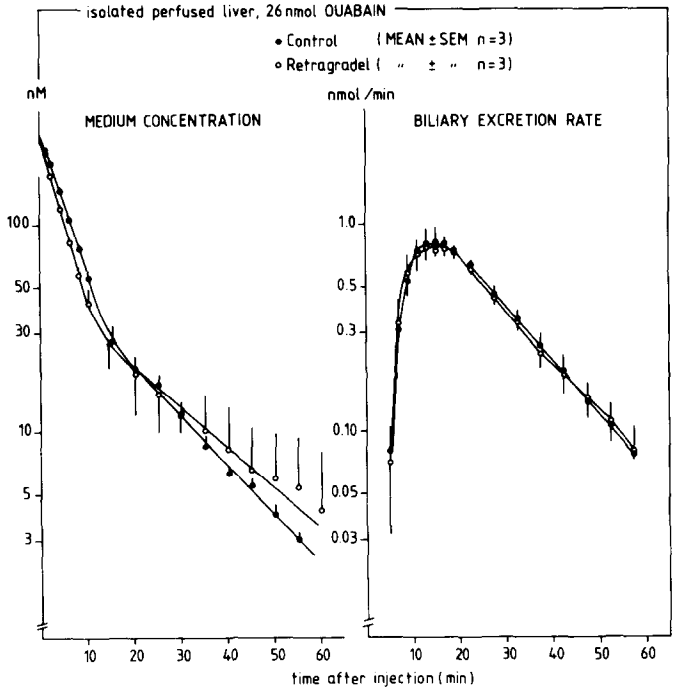

Fig. 6. Medium disappearance (left panel) and biliary excretion rate (right panel) of 26 nmole [^{3}H]ouabain administered to isolated perfused livers with normal (●) and retrograde (○) direction of perfusate flow.

Table 2. Hepatic transport of 26 nmole ouabain in normal and retrograde perfusions

	Normal ($n = 3$)	Retrograde ($n = 3$)
$t_{\frac{1}{2}}\alpha$ (min^{-1})	3.5 ± 0.2	2.8 ± 0.2
$t_{\frac{1}{2}}\beta$ (min^{-1})	17.9 ± 3.0	20.0 ± 1.6
Cl (ml/min)	12.3 ± 0.3	13.7 ± 1.3
E (%)	32.5 ± 0.4	43.1 ± 1.0*
Bile flow (μl/min)	10.3 ± 0.5	12.5 ± 0.9
Liver weight (g)	9.5 ± 0.7	9.4 ± 1.0

The plasma disappearance rate of 26 nmol [^{3}H]ouabain was measured in normal and retrograde perfusions. The curves were fitted according to a biexponential equation and the half-lifes of the first ($t_{\frac{1}{2}}\alpha$) and the second phase ($t_{\frac{1}{2}}\beta$) are given. The clearance (*Cl*) was calculated from the dose and the area under the plasma disappearance vs time curve. The extraction (*E*) was calculated from the amount of radioactivity present in the medium 1 min after injection, sampled before (C_{in}) and after (C_{out}) the liver. All values are mean ± S.E.

* Significantly different from normal perfusion, $P < 0.05$.

initially steeper when $k_5 = 99\ k_6$, but after about 60 min the curves became parallel again. These results indicate that only if k_1 and k_3 are very high, resulting in a preferential accumulation in zone 1 in normal perfusions and in a preferential accumulation in zone 3 in retrograde perfusions, differences in k_5 and k_6 may be detected in the biliary excretion rate curves.

To predict the concentration in zones 1 and 3 of the liver at 2 and 10 min after injection, these concentrations were calculated in the above mentioned simulated experiments and the ratio of these concentrations are given in Table 3. It is evident that only when the plasma to liver transport rate is different between the two zones, the autoradiographs will show clear gradients of the studied compound. However, when zonal differences in liver to bile or liver to plasma clearance constants were simulated, the zone 1:zone 3 concentration ratio varied only between 0.6 and 1 at these timepoints. In these cases, more pronounced concentration differences in the zones occurred only later than about 40 min after injection.

DISCUSSION

(D)BSP

In this study DBSP was used in the perfusion experiments to determine the transport rate constants, whereas BSP was used for the autoradiography. Although BSP is metabolized in the rat liver by conjugation to glutathione, BSP and DBSP

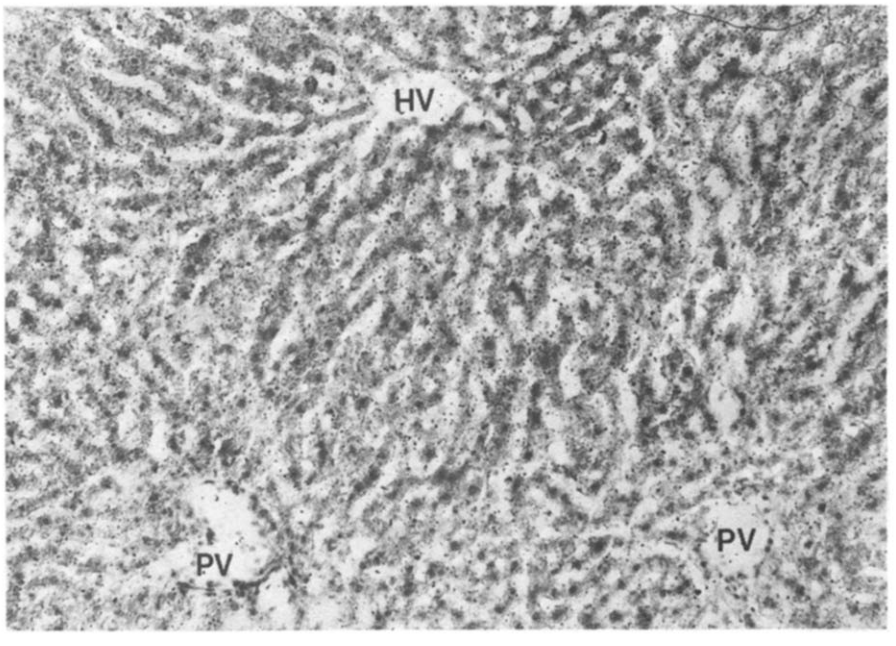

Fig. 7. Autoradiograph 30 sec after injection of 1 nmole of [^{3}H]ouabain in the inflow cannula of a normally perfused liver. Grains are homogeneously distributed in the acinus. The same distribution was seen in retrogradely perfused livers. PV, portal venule; HV, terminal hepatic venule (144×).

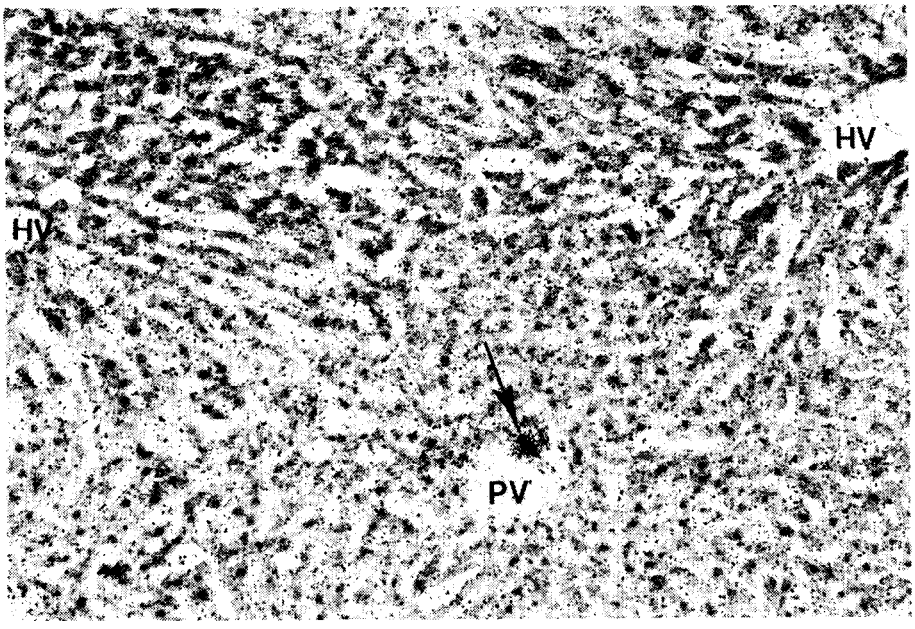

Fig. 8. Autoradiograph 10 min after injection of 1 nmole of [^{3}H]ouabain to a normally perfused liver. Grains are homogeneously distributed in the acinus and bile ducts are labelled (arrow). The same distribution pattern was found 10 min after injection to retrogradely perfused livers. PV, portal venule; HV, terminal hepatic venule (144×).

are assumed to be taken up by the same transport mechanism and the plasma disappearance rates for BSP and DBSP in the rat are similar [15]. Moreover, 2 min after injection the influence of metabolism on the concentrations of the label in the two liver zones can be neglected.

The autoradiographs of livers 2 min after injection of [^{35}S]BSP showed a homogeneous tissue labelling in the acinus (Fig. 4). Since the initial extraction was about 35–50%, no steep concentration gradients can be expected in the sinusoidal blood. The data from the computer simulations (Table 3) showed that the concentration of the label in the two liver zones at this timepoint should be markedly different when the uptake rates are different. Since a concentration ratio of about 1.5–2 should be readily detected on autoradiographs even without grain counting, it can be concluded that if zone 1 and zone 3 cells differ

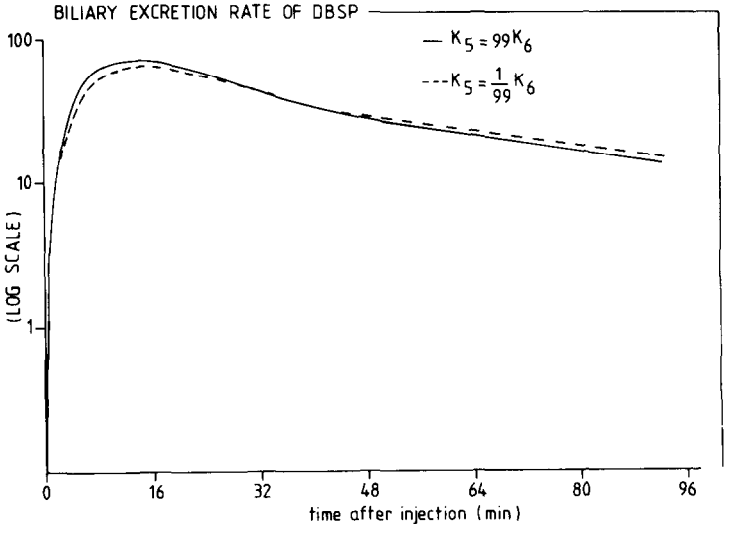

Fig. 9. Biliary excretion rate of DBSP in normal (——) and retrograde (– – –) perfusion, calculated by computer simulation for livers with acinar heterogeneity with respect to biliary excretion rate, according to the model described in Methods. The simulation for the normal perfusion was performed with $k_1 = k_3$, $k_2 = k_4$ and $k_5 = 99\ k_6$; the simulation for the retrograde perfusion with $k_1 = k_3$, $k_2 = k_4$ and $k_5 = 1/99\ k_6$. The biliary excretion rate is expressed as arbitrary units of DBSP per min.

Table 3. Ratio of DBSP concentrations in zones 1 and 3, 2 and 10 min after injection, calculated by computer simulations for normal and retrograde perfusions.

Rate constants			Concentration 'zone 1':'zone 3' (2 min)		(10 min)	
Plasma to liver	Liver to plasma	Liver to bile	Normal	Retrograde	Normal	Retrograde
$k_1 = k_3$	$k_2 = k_4$	$k_5 = k_6$	1.12	0.89	1.12	0.89
$k_1 = 3k_3$	$k_2 = k_4$	$k_5 = k_6$	3.3	2.5	3.2	2.5
$k_1 = 9k_3$	$k_2 = k_4$	$k_5 = k_6$	9.6	6.9	9.2	7.1
$k_1 = 99k_3$	$k_2 = k_4$	$k_5 = k_6$	98	70	97	78
$k_1 = k_3$	$k_2 = 3k_4$	$k_5 = k_6$	1.04	0.78	0.96	0.75
$k_1 = k_3$	$k_2 = 9k_4$	$k_5 = k_6$	1.04	0.76	0.90	0.67
$k_1 = k_3$	$k_2 = 99k_4$	$k_5 = k_6$	1.02	0.76	0.82	0.63
$k_1 = k_3$	$k_2 = k_4$	$k_5 = 3k_6$	1.00	0.78	0.94	0.72
$k_1 = k_3$	$k_2 = k_4$	$k_5 = 9k_6$	1.04	0.78	0.81	0.63
$k_1 = k_3$	$k_2 = k_4$	$k_5 = 99k_6$	1.05	0.83	0.75	0.58

The ratio's of the DBSP concentrations at 2 and 10 min after injection in normal and retrograde perfusions are calculated by computer simulations as given in Fig. 1 and in Methods. The ratio's are calculated assuming that the rate constants in 'zone 1' are higher than in 'zone 3'. For the opposite simulation the column headings 'normal' and 'retrograde' should be exchanged.

in uptake rate, this will be <50%. In contrast, Table 3 also shows that the concentrations in the two zones will differ only slightly 2 min after injection, even when the clearance constants for liver to bile (k_5 and k_6) or liver to plasma (k_2 and k_4) transport would be 100 times higher in one of the zones compared to the other zone. With the present autoradiographic technique these small differences are unlikely to be detected. Also 10 min after injection, when the calculated concentrations would differ more between the zones, these differences might be hard to detect experimentally, even when grain-numbers are quantitated by counting. Under optimal conditions a 20% difference in concentration of label is the detection limit for grain counting [18]. Therefore, to investigate whether zone 1 and zone 3 cells are heterogeneous with respect to the transport rates out of the cells a more quantitative analysis of the autoradiographs taken a longer time after injection would be necessary. However, it might be speculated that the higher labelling of zone 3 occasionally observed in autoradiographs both after normal and retrograde perfusions indicates that the zone 1 cells are better equipped for biliary excretion, or have a higher liver to plasma transport rate. It should be stressed again here, that, as indicated by the computer simulations, marked zonal differences in the transport processes out of the cells, would only result in relatively small gradients. Indications for a predominantly periportal localization of the biliary excretion of DBSP was also obtained after selective zone 3 damage by carbon tetrachloride [10]. Alternatively, a slightly higher uptake rate may be present in zone 3, but this is not in line with the results obtained after selective acinar damage [10].

Slight differences were found in the plasma disappearance and biliary excretion rates for DBSP between normal and retrograde perfusions both with and without bile salt infusion. The computer simulations showed that irrespective of a heterogeneous distribution of transport functions, no changes whatsoever in the plasma disappearance occur upon reversal of the flow through the liver. In contrast, the biliary excretion rate curve may change moderately upon reversal of the flow. Therefore the combined results, showing differences in both the plasma disappearance and biliary excretion rate curves between the normal and retrograde perfusions, cannot be explained by a heterogeneous localization of DBSP transport in the liver acinus. In the perfusions without bile salt infusion, the higher k_{12}, k_{23} and bile blow can at least partly be explained by the higher liver weight in this particular group of rats. This higher liver weight was not due to the retrograde perfusion, because in all other experiments [DBSP without bile salts (Table 1) ouabain with bile salts (Table 2) and taurocholate [13]], the liver weights were the same after normal or retrograde perfusions. When correction was made for the higher liver weight, k_{12} and k_{23} were still slightly higher in the retrograde perfusions, but the differences were not statistically significant. However, a small but significant difference between normal and retrograde perfusions was found in k_{23} when a bile salt infusion was given. In this case liver weights were equal in both types of perfusion (Table 1). Therefore, the higher biliary excretion rate in retrograde perfusions may be ascribed to indirect effects of the retrograde perfusion flow. For instance in the normal perfusions the highest oxygen [1] and bile salt concentrations [8, 13] are present in zone 1, whereas in retrograde perfusions the highest concentrations of the these compounds are present in zone 3 [13]. Zone 3 cells might be more sensitive to stimulation of DBSP transport by bile salts [19] or oxygen than zone 1 cells.

Ouabain

The autoradiographic experiments with ouabain showed that ouabain is uniformly distributed in the acinus. This indicates that no major difference in uptake rate exists between the acinar zones. Due to this lack of a clear zonal gradient, it is not feasible to make conclusions concerning the relative involvement of zones 1 and 3 in the biliary excretion of ouabain (as the computer simulations showed).

No differences were found in the plasma disappearance and biliary excretion rate for normal and retrograde perfusions. Only the hepatic extraction 1 min after injection was significantly higher in the retrograde perfusions. As the computer simulations showed, this cannot be explained by a heterogeneous localization of the ouabain uptake process, but should rather be ascribed to secondary effects of the reversed flow. Ouabain transport is not influenced by bile salts [20] and no protein binding occurs in plasma or liver [17]. But since ouabain is taken up by secondary active transport [16, 21] the reversed oxygen gradient might influence liver uptake.

The experiments of Reuning and Schanker [17], who studied ouabain transport after CCl_4 intoxication and observed a marked reduction in biliary excretion rate, might in principle indicate that a considerable amount of ouabain is normally excreted by zone 3 cells. However, previous studies [22] revealed that not only zone 3 cells but also zone 1 cells might be damaged with the dose of CCl_4 (1 ml/kg) used by Reuning and Schanker.

CONCLUSIONS

Our experiments indicate that periportal and perivenous cells are more or less equally involved in (D)BSP and ouabain uptake from the plasma. Computer simulations showed that provided that all liver cells are equally involved in the hepatic uptake, a zonal heterogeneity in biliary transport rate is not clearly reflected in changes in the plasma disappearance and biliary excretion rate curves upon reversal of the perfusate flow. Thus, the present results cannot definitely elucidate the relative involvement of zone 1 and zone 3 cells in biliary excretory function with respect of DBSP and ouabain. However, such a possible difference in excretory function between these cells is certainly not excluded by our data. In any event the similar kinetic patterns of both DBSP and ouabain during normal and retrograde perfusions indicate that the latter technique does not lead to a major deterioration of the liver transport functions in general.

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