Influence of dehydrocholate and taurocholate on bromsulphthalein uptake, storage, and excretion in the dog

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SUMMARY The influence of dehydrocholate on bromsulphthalein relative-storage capacity, biliary transport maximum (Tm), and fractional transfer rates between plasma, liver, and bile have been studied in unanaesthetized dogs. In six dogs, storage capacity, Tm, and fractional transfer rates from plasma to liver, liver to bile, and liver to plasma were measured during 0.15 M NaCl infusion and the measurements were repeated under a dehydrocholate infusion of 95 $\mu\text{mol}\cdot\text{min}^{-1}$, ie, an infusion rate approaching the known biliary Tm of bile salts. It was found that: (a) storage capacity and fractional transfer rates from plasma to liver were significantly lower during dehydrocholate infusions (respectively $18.0 \pm \text{SD } 9.0 \text{ mg}\cdot\text{mg}^{-1}\cdot 100 \text{ ml}^{-1}$ and $0.120 \pm \text{SD } 0.035 \text{ min}^{-1}$) than during NaCl infusions (respectively $47.0 \pm 21.0 \text{ mg}\cdot\text{mg}^{-1}\cdot 100 \text{ ml}^{-1}$ and $0.280 \pm \text{SD } 0.055 \text{ min}^{-1}$; $P < 0.001$); (b) Tm and fractional transfer rates from liver to bile were also significantly lower during dehydrocholate infusions (respectively $3.2 \pm \text{SD } 1.1 \text{ mg}\cdot\text{min}^{-1}$ and $0.013 \pm \text{SD } 0.004 \text{ min}^{-1}$) than during NaCl infusions ($4.8 \pm \text{SD } 1.1 \text{ mg}\cdot\text{min}^{-1}$ and $0.033 \pm \text{SD } 0.017 \text{ min}^{-1}$; $P < 0.02$); (c) in three additional experiments, taurocholate had similar effects on storage capacity and Tm. These findings suggest that competition occurred between bile salts and bromsulphthalein for hepatic uptake and storage. They support the hypothesis that the decreased disappearance rate and relative storage capacity of bromsulphthalein observed during biliary obstruction may be due to competition between bile salts and bromsulphthalein for hepatic uptake and storage.

Hepatobiliary elimination of bromsulphthalein (BSP) involves three major stages; hepatocellular uptake, intracellular transport (including conjugation and storage), and excretion into bile canaliculi (Levy, 1961; Javitt, 1970). Measurement of relative storage capacity and maximal biliary excretion (transport maximum or Tm) provides a means of assessing quantitatively the storage function of the hepatocytes and their capacity for biliary excretion (Wheeler, Epstein, Robinson, and Snell, 1960a; Wheeler, Meltzer, and Bradley, 1960c). It had been anticipated that the relative storage capacity would be predominantly decreased in hepatocellular disease, whereas Tm would be more specifically altered in disorders of biliary function (Wheeler *et al*, 1960c); thus simultaneous measurement of storage capacity and Tm would have provided a

means of distinguishing between hepatic parenchymal disease and biliary obstruction or intrahepatic cholestasis. In practice, such distinction has not been possible because storage capacity and Tm are generally both decreased in parenchymal disease and cholestasis (Wheeler *et al*, 1960c). The reason why storage capacity is substantially decreased during cholestasis (especially during biliary obstruction) has never been satisfactorily explained. It is known that competition may occur for hepatic uptake (and therefore, possibly, storage) between bile acids and BSP (Bourdon, Fauvert, Nicollo, and d'Auteuil, 1960; Wheeler *et al*, 1960c). Since serum bile acid concentration is increased during biliary obstruction, these studies were undertaken to test the hypothesis that the decrease in storage capacity observed during biliary obstruction could be related to competition between bile acids and bromsulphthalein. The results indicate that the infusion of bile acids significantly decreases both BSP storage capacity and BSP transfer rate from plasma to liver.

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Material and Methods

EXPERIMENTAL PROTOCOLS

Two types of experiments were performed.

1 Measurement of BSP, Tm, and storage capacity (S)
Bromsulphthalein Tm and S were measured using the double-infusion technique of Wheeler *et al* (1960c) in six unanaesthetized mongrel dogs weighing 16 to 23 kg. In each dog, two measurements were performed, the first with an intravenous infusion of 0.15 M NaCl (control experiment), the second, at least three weeks later, with an intravenous infusion of sodium dehydrocholate in H₂O (Theraplix Laboratories, Paris, France) at a rate of 95 $\mu\text{mol}\cdot\text{min}^{-1}$ (group A). In addition, in order to compare the influence of dehydrocholate on storage capacity and Tm to that of taurocholate, storage capacity and Tm were measured in three dogs (group B) with a sodium taurocholate infusion. Two of these dogs (G and H) had already been investigated with dehydrocholate in group A. Sodium taurocholate (Sigma, St Louis, Missouri, USA), in glucose 50 g/l: Na₂CO₃ 0.1 M, 1:1, v/v was infused at a rate of 110 $\mu\text{mol}\cdot\text{min}^{-1}$. The volume of water infused was 0.2 ml. min^{-1} in the three groups of experiments. In each case, the bile salt infusion was started 30 min before the BSP infusion. The rate of bile salt infusion was chosen to approximate the known maximal rate of taurocholate excretion into bile (Wheeler, Mancusi-Ungaro, and Whitlock, 1960b; O'Maille, Richards, and Short, 1965). Dehydrocholate was used because, in contrast to taurocholate, it does not produce haemolysis at high infusion rates. At the end of each experiment, plasma volume was measured using a standard dilution method with ¹²⁵I-labelled human serum albumin (Centre National de Transfusion Sanguine, Paris, France).

2 Compartmental analysis of BSP disappearance curve

In the six dogs of group A, after a single intravenous injection of 5 mg/kg body weight⁻¹ of BSP, blood

samples were taken at two, four, six, nine, 12, 16, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, and 90 min and plasma BSP concentration was measured. Again, in each dog, two experiments were performed, the first under a continuous intravenous infusion of 0.15 M NaCl at a rate of 0.2 ml. min^{-1} (control experiment), the second under an intravenous infusion of sodium dehydrocholate, at a rate of 95 $\mu\text{mol}\cdot\text{min}^{-1}$, in a volume of 0.2 ml. min^{-1} . The infusions of NaCl or dehydrocholate were started 30 min before the injection of BSP.

ANALYTICAL PROCEDURES AND CALCULATIONS

Bromsulphthalein concentration in plasma was measured after appropriate dilution in KH₂PO₄ 0.15 M: Na₂HPO₄ 0.11 M, 4:7, v/v buffer, pH 6.7, after alkalization with K OH 3.6 N in a Zeiss PM Q2 spectrophotometer at 580 nm. The plasma disappearance curve of BSP was subjected to compartmental analysis (Barber-Riley, Goetzee, Richards, and Thomson, 1961) using a two-compartment model system and a conventional graphic technique. The graph of the concentration at time t (Ct) may be described in this model as the sum of two exponential functions; these two functions are respectively Ae^{- μ t} and Be^{- ν t}. The plasma BSP concentration at time t is: Ct = Ae^{- μ t} + Be^{- ν t}. The fractional transfer rates were calculated according to the following formulae (Barber-Riley *et al*, 1961): Plasma to liver transfer rate: k₂₁ = (A μ + B ν)/(A + B)

Liver to bile transfer rate: k₃₂ = $\mu\nu/k_{21}$

Liver to plasma transfer rate: k₁₂ = (μ + ν) - (k₂₁ + k₃₂)

The transfer rate from bile to liver is assumed to be negligible (k₂₃ $\approx$ 0). The comparison of means was performed with the Student's t test for paired data.

Results

The influence of dehydrocholate on BSP Tm and storage capacity in the dogs of group A is indicated

Dog	Storage Capacity (mg.mg ⁻¹ .100 ml ⁻¹)		Biliary Transport Maximum (mg.min ⁻¹)	
	Control	Dehydrocholate	Control	Dehydrocholate
A	70.0	29.0	4.5	3.8
B	72.0	23.0	6.0	4.7
C	36.0	10.0	3.8	2.8
D	37.0	12.0	6.3	2.6
E	18.0	7.5	4.1	1.6
F	41.0	27.0	4.1	3.9
Mean	47.0	18.0	4.8	3.2
SD	21.0	9.0	1.1	1.1
P	<0.001		<0.02	

Table I Influence of dehydrocholate on BSP relative storage capacity and biliary transport maximum in the dog

Dog	BSP Relative Storage Capacity (mg.mg ⁻¹ .100 ml ⁻¹)		Biliary Transport Maximum (mg.min ⁻¹)	
	Dehydrocholate	Taurocholate	Dehydrocholate	Taurocholate
G ¹	12.0	3.5	2.6	3.3
Y ¹	7.5	0.0	1.6	2.2
I	31.0	22.0	2.7	2.9
Mean	17.0	8.5	2.3	2.8

Table II Comparison of the influence of dehydrocholate and taurocholate on BSP relative storage capacity and biliary transport maximum in three dogs¹

¹Dogs G and H are also dogs D and E of group A

Dog	k_{21} (mg.mg ⁻¹ .min ⁻¹)		k_{12} (mg.mg ⁻¹ .min ⁻¹)		k_{32} (mg.mg ⁻¹ .min ⁻¹)	
	Control	Dehydrocholate	Control	Dehydrocholate	Control	Dehydrocholate
A	0.288	0.109	0.020	0.019	0.026	0.009
B	0.351	0.167	0.008	0.019	0.050	0.016
C	0.280	0.140	0.025	0.024	0.051	0.009
D	0.307	0.110	0.020	0.026	0.044	0.016
E	0.183	0.063	0.013	0.071	0.017	0.018
F	0.270	0.136	0.010	0.020	0.012	0.011
Mean	0.280	0.120	0.016	0.030	0.033	0.013
SD	0.055	0.035	0.007	0.020	0.017	0.004
P	<0.001		NS		<0.02	

Table III Influence of dehydrocholate on BSP transfer rates from plasma to liver (k_{21}), from liver to plasma (k_{12}), and from liver to bile (k_{32}) in the dog

in table I. Values for storage capacity were significantly lower when a dehydrocholate infusion was administered than in the controls. The mean decrease was 61%. The Tm was also significantly reduced when dehydrocholate was infused compared with controls: the mean decrease was 33%. A comparison between the influence of dehydrocholate and taurocholate in the three dogs of group B appears in table II. Storage capacity was lower in taurocholate-infused than in dehydrocholate-infused animals. The influence of both bile salts on Tm was similar.

The influence of dehydrocholate on BSP transfer rates is indicated in table III. The plasma to liver transfer rate (k_{21}) was significantly lower under dehydrocholate infusion than in the controls. The mean decrease was 57%. The liver to bile transfer rate (k_{32}) was also significantly lower under dehydrocholate infusion than in controls. The mean decrease was 61%. The liver to plasma transfer rate (k_{12}) was not significantly different in the two groups.

Discussion

The results indicate that a dehydrocholate infusion causes a parallel decrease in BSP storage capacity, and BSP transfer rate from plasma to liver; in addition, the BSP Tm and transfer rate from liver to bile are both reduced.

The reductions in storage capacity and the transfer rate from plasma to liver could be due either to

diminished transfer through the sinusoidal membrane or to a decrease in binding to the cytoplasmic proteins Y and Z (Levi, Gatmaitan, and Arias, 1969) or both. The available evidence suggests that bile salts are not bound to the Y protein: for instance, taurocholate did not displace BSP from the Y protein *in vitro* (Levi *et al.*, 1969; Litwack, Ketterer, and Arias, 1971). Although most of the experiments in this work were performed with dehydrocholate, a semi-synthetic triketocholanoic bile salt, the results obtained with taurocholate were qualitatively similar. If the effect of bile salts on BSP storage capacity and transfer rate is not due to interference with Y binding, it might be due to interference with transport through the sinusoidal plasma membrane. It has already been reported that dehydrocholate decreases the plasma to liver transfer rate of rose bengal (Kelman-Sraer, Erlinger, Peignoux, and Benhamou, 1973), a phthalein dye structurally related to BSP, as well as BSP uptake (Andrews and Richards, 1960; Wheeler *et al.*, 1960c). Bromsulphthalein 'carriers' in the liver cell membrane have been postulated (Frezza, Tiribelli, Panfili and Sandri, 1974). A taurocholate-binding protein has recently been identified in isolated liver cell membranes (Accatino and Simon, 1974) and taurocholate transport through the liver cell membrane has been postulated to be carrier mediated (Glasinović, Dumont, Duval, and Erlinger, 1973; Reichen and Paumgartner, 1973). The results of these experiments

suggest that the transport of bile salts and BSP across the liver cell membrane may share a common step. Whatever the molecular mechanism involved, these findings suggest that the decrease in BSP disappearance rate and relative storage capacity (Wheeler *et al.*, 1960c) observed during biliary obstruction could be due, as in our experiments, to an increased plasma bile salt concentration. Although bile salt concentration in blood has not been measured in our experiments, it is likely to be elevated when bile salts are infused at a rate approaching Tm (Wheeler *et al.*, 1960b; O'Maille *et al.*, 1965).

The second group of findings is the decrease of BSP Tm and transfer rate from liver to bile. This is apparently in contrast to the increase in Tm observed in several species, including the dog, during taurocholate (Ritt and Combes, 1967; Gibson and Forker, 1972; Barnhart, Ritt, Ware, and Combes, 1973; Erlinger and Dumont, 1973) or dehydrocholate infusions (Bourdon *et al.*, 1960; Ritt *et al.*, 1967). However, in all these experiments, bile salt infusion was started after BSP infusion. In our experiments, as well as in experiments in rats (Bourdon *et al.*, 1960), dehydrocholate was administered before BSP; it is conceivable that under these conditions, because of the interference with uptake discussed above, the input of BSP into the liver is insufficient for the Tm to be increased, or even maintained. In addition, it should be pointed out that a reduction in Tm has also been observed during prolonged infusion of bile salts in the dog (Barnhart *et al.*, 1973; Gibson and Forker, 1974). Finally, it is noteworthy that the rose bengal transfer rate from liver to bile was increased by dehydrocholate (Kelman-Sraer *et al.*, 1973), whereas, in our experiments, the BSP transfer rate was decreased. The reason for this discrepancy is unknown; it could be due to the existence of two separate carriers for the transport into bile of BSP and rose bengal; another argument for this hypothesis is that phenobarbital, which increases BSP Tm in the rat, does not increase rose bengal Tm (Berthelot, Dhumeaux, and Préaux, 1969).

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