Kinetic Measurements of the Biliary Excretion of Metabolized Compounds

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1. Rats received constant infusions of bromosulphophthalein or [*carboxy*-¹⁴C]cholic acid at a range of concentrations. 2. Kinetic parameters describing the biliary excretion of the compounds were determined. 3. The biliary excretion of both compounds could be described by the same kinetic parameters already calculated for phenolphthalein disulphate, which is excreted in the bile unchanged.

Kinetic parameters describing the biliary excretion of phenolphthalein di[³⁵S]sulphate in the rat have been reported previously (Powell *et al.*, 1975). It was shown that kinetic evaluation of the data obtained with this ester was consistent with the model system described by:

where k is the infusion rate of the ester, A is the amount of the ester in the animal having direct access to the bile and to an alternative compartment, k_1 is the rate constant for the biliary excretion of the ester, B is the amount of ester in the bile, k_2 is the rate constant for entry into the alternative compartment and C represents the amount of ester in that compartment.

The present study was carried out to determine whether this model system could be applied to other compounds both naturally-occurring and xenobiotic, which are eliminated in the bile. In particular, it was of interest to apply the model to compounds that, unlike phenolphthalein disulphate, are metabolized in the liver before appearing in the bile.

Experimental and Results

Bromosulphophthalein was obtained as the sodium salt from Sigma (London) Chemical Co., Kingston, Surrey, U.K. [*carboxy*-¹⁴C]Cholic acid, sodium salt (sp. radioactivity 51 Ci/mol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Experimental animals

Male M.R.C. hooded rats (approx. 300g body

wt.) were used throughout. Animals were anaesthetized with diethyl ether, the right jugular vein was cannulated and pentobarbitone sodium (Nembutal; Abbott Laboratories, Queenborough, Kent, U.K.) was administered via the cannula at a dose of 10–20 mg/kg body wt. Animals were maintained under anaesthesia with similar doses of Nembutal as necessary throughout the experiments. After a mid-line abdominal incision the common bile duct was cannulated. The left jugular vein was cannulated also and continuous infusions were introduced via this cannula.

Measurement of radioactivity in bile

Samples (10 μ l) of bile were added to 10 ml of scintillation 'cocktail' [1600 ml of toluene, 400 ml of methyl oxitol [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] and 14 g of 2,5-diphenyloxazole (scintillation grade)]. The vials were counted for radioactivity with a Tracerlab Corumatic 200 liquid-scintillation spectrometer. Sufficient counts were recorded to give a standard error of $\pm 2\%$.

Measurement of bromosulphophthalein in bile

Bile samples (10, 50 or 100 μ l, to provide a suitable absorbance) were added to 10 ml of water and 1 ml of NaOH (0.1 M) and the absorbance was measured at 580 nm with a Cecil CE 373 spectrophotometer. Absorbance values were read against a calibration curve for bromosulphophthalein, which was linear over the concentration range 0.4–40 μ g/ml.

Animals with bile-duct cannulae received continuous infusions of either bromosulphophthalein or ¹⁴C-labelled sodium cholate at a rate of 15 μ l/min (Unit 1 Continuous Infusion Apparatus; B. Braun, Melsungen, Germany). The concentrations of the solutions were adjusted so that each substance was infused at the following rates: 3.0, 4.5, 9.0 and 12.0 μ g/min. In all experiments, bile samples were

collected in tared tubes at regular intervals (usually 15 min) for 3–4 h after the beginning of the infusion. Bile weights were recorded and in experiments with ^{14}C -labelled sodium cholate each bile sample was assayed for total radioactivity. In experiments with bromosulphophthalein, bile samples were assayed spectrophotometrically.

When bromosulphophthalein or ^{14}C -labelled cholic acid was infused at a constant rate, the rate of excretion increased with time, reaching a limiting value that was then maintained for the duration of the experiment. For both compounds there was an initial short lag period before steady-state was achieved and thus the steady-state curves do not extrapolate to zero. With the two compounds at each infusion rate the rate of excretion was less than the corresponding infusion rate. The excretory mechanism was not saturated for either of the compounds studied and a steady-state with respect to biliary excretion was achieved in each case. A typical set of progress curves is shown in Fig. 1.

Fig. 1. Biliary excretion of ^{14}C in rats receiving continuous intravenous infusions of [carboxy- ^{14}C]cholate. The compound was infused at the following rates ($\mu\text{g}/\text{min}$): $\bullet$, 3.0; $\blacksquare$, 4.5; $\square$, 9.0; $\circ$, 12.0.

Fig. 2. Relationship between the rate of infusion of the compound being investigated and the steady-state rate of its excretion in bile

(a) Bromosulphophthalein; (b) ^{14}C -labelled cholate.

The steady-state portion of the curves is described by the expression:

$$B = Skt - Y$$

where k is the infusion rate, B is the amount of test substance in the bile, Y is a composite constant and t is time. The slope at the steady-state gives S (the proportion of the infused compound excreted in bile at steady-state) and this value was calculated for the two compounds at each infusion rate. The values obtained are recorded in Table 1. Analysis of the values recorded in Table 1 showed no positive

correlation between bile production and the rate of infusion. Further, in the experiments with both bromosulphophthalein and ^{14}C -labelled cholate, no correlation existed between the rate of bile formation and the value S . With the two compounds a positive correlation was shown between the rate of excretion of the infused compound during the steady-state and the rate of infusion (Fig. 2).

Discussion

The two-compartment open model used to de-

scribe the distribution and excretion of phenolphthalein disulphate (Powell *et al.*, 1975) can also be applied to the two compounds studied in the present paper. It is particularly interesting that although the two compounds (bromosulphophthalein and cholate) are known to be extensively metabolized in the liver, the kinetics of biliary excretion during continuous infusions of either appear identical with those described for phenolphthalein disulphate, which is excreted in the bile unchanged (Millburn, 1970). These results are consistent with the reaction sequences shown in Scheme 1.

Table 1. *Biliary excretion of two compounds administered to rats by continuous intravenous infusion*
For further details see the text. The results in parentheses in the last column are means $\pm$ S.D.

Compound	Infusion rate ($\mu\text{g}/\text{min}$)	Mean bile flow ($\mu\text{l}/15\text{ min}$)	Rate of excretion at steady-state ($\mu\text{g}/\text{min}$)	S
Bromosulphophthalein	3.0	332	2.25	0.75
	4.5	254	4.37	0.97
	9.0	259	7.65	0.85
	12.0	303	10.68	0.89
				(0.865 $\pm$ 0.091)
[carboxy- ^{14}C]Cholate	3.0	358	2.49	0.83
	4.5	308	3.56	0.79
	9.0	325	8.10	0.90
	12.0	340	11.16	0.93
				(0.863 $\pm$ 0.045)

Scheme 1. *Model system representing the fate of infused compounds and any metabolite(s) formed in vivo*

k is the infusion rate of the compound being investigated, A represents the amount of the compound in a volume V_A having direct access to bile, a second volume V_C and a metabolic pathway leading to metabolite M . B , C , M and MB represent the amounts of the compound in bile and compartment C and the amount of metabolite in the animal and the bile respectively. k_1 , k_2 , k_3 and k_4 are apparent first-order rate constants for the steps indicated. In particular, k_2 may be a composite constant that nonetheless describes the irreversible removal of material from A . This is so irrespective of the number of steps between A and several possible alternative compartments collectively designated as C . Neither the possible nature of k_2 nor the number of steps between A and C will affect the kinetics at steady-state.

During the steady-state period, the rate of excretion of unchanged compound and its metabolites is given by eqns. (1) and (2):

$$\frac{dB}{dt} = \frac{k_1 k}{(\alpha + k_3)} \quad (1)$$

and

$$\frac{d(MB)}{dt} = \frac{k_3 k}{(\alpha + k_3)} \quad (2)$$

where

$$\alpha = (k_1 + k_2)$$

The slopes of the steady-states shown in Fig. 1 are given by the sum of eqns. (1) and (2). Hence the proportion of the infused compound excreted during this period is given by S :

$$S = (k_1 + k_3)/(\alpha + k_3) \quad (3)$$

If there is irreversible removal of M with an overall rate constant k_s , then eqn. (3) becomes:

$$S = \frac{k_1(k_4 + k_s) + k_3 k_4}{(\alpha + k_3)(k_4 + k_s)} \quad (4)$$

The comments made with respect to k_2 are also applicable to k_s . Thus, k_s may also represent a composite rate constant involving a number of steps, but overall it describes the irreversible removal of material (in this case metabolized material) to a compartment, comparable with compartment C, from which biliary excretion cannot take place. In both eqns. (3) and (4), the values for S will range from zero to 1 and reflect the quantitative importance of biliary excretion of the compound and its metabolite(s) compared with secretion into alternative compartments, which are collectively referred to as C in Scheme 1. The nature and location of compartments C are obviously of interest, but are unknown at present and may be different for different compounds. They could, for example, represent different excretion routes. However, another important consideration is the rate of accumulation of the compound and its metabolite(s) in compartments such as C because this competes directly with the biliary excretion mechanism. It is the extent of this competition that is accurately measured by S , the rate of excretion into bile during the steady-state periods illustrated in Fig. 1.

The proportion (S) of the infused compound that is excreted during the steady-state is a precise

measurement of the distribution of the compound and its metabolite(s) between bile and any other compartment such as C. It is pertinent to note that the mean values for S determined for bromosulphophthalein and cholate are not significantly different from each other, but they are probably different from values previously recorded for phenolphthalein disulphate ($P = 0.07$). Since S is a constant that describes the distribution of a compound and its metabolite(s) between the biliary and other compartments, it may well be an important value to determine for other compounds that are excreted to various extents in the bile.

There are two major possible approaches to the question of structural requirements of molecules in relation to biliary excretion. One approach is to relate the extent of biliary excretion of administered compounds to their molecular features. This approach has shown that certain physicochemical parameters (molecular weight, polarity, molecular structure) markedly influence the extent to which a compound is excreted into bile (Millburn *et al.*, 1967; Hirom *et al.*, 1972) and it has been suggested that it is possible to predict from the characteristics whether bile or urine will be the major excretory route for a particular compound (Hirom *et al.*, 1976). However, it is clear that the biliary excretion of a compound is a multi-stage process and the passage of a choleophile from the hepatocyte into the bile is just one of them. The second major approach to the question of biliary excretion must seek to delineate the different mechanisms involved. In this approach, the values for S in relation to physical and chemical parameters may be more useful than the usually quoted percentage excretion values obtained with animals receiving single doses of substances.

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