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# The Disposition of Aspirin and Salicylic Acid in the Isolated Perfused Rat Liver: the Effect of Normal and Retrograde Flow on Availability and Mean Transit Time

GEORGE D. MELLICK AND MICHAEL S. ROBERTS

*Department of Medicine, The University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia*

## Abstract

The effect of changing the direction of perfusate flow from anterograde to retrograde on the disposition of acetylsalicylic acid (aspirin) and salicylic acid was studied in the single pass in-situ perfused rat liver. Mixtures of aspirin, [$^{14}$C]salicylic acid and the inert reference solute [$^{3}$H]sucrose were administered as boluses into the liver using red blood cell and albumin-free perfusate media at a flow rate of 30 mL min$^{-1}$/liver. Hepatic availability (F), mean transit time (MTT) and normalized variance ($CV^2$) for aspirin, preformed [$^{14}$C]salicylic acid, salicylic acid produced from aspirin in the liver and [$^{3}$H]sucrose were deduced from the outflow concentration profiles using statistical moment analysis.

The values for F, MTT and $CV^2$ for the solutes under anterograde perfusion were: aspirin ($0{\cdot}73 \pm 0{\cdot}04$, $15{\cdot}13 \pm 2{\cdot}01$ s, $0{\cdot}33 \pm 0{\cdot}09$, $n = 5$), preformed [$^{14}$C]salicylic acid ($1{\cdot}05 \pm 0{\cdot}06$, $n = 12$, $43{\cdot}19 \pm 2{\cdot}21$ s, $1{\cdot}08 \pm 0{\cdot}08$, $n = 5$), salicylic acid from aspirin ($0{\cdot}33 \pm 0{\cdot}05$, $42{\cdot}82 \pm 9{\cdot}16$ s, $0{\cdot}73 \pm 0{\cdot}10$, $n = 5$) and [$^{3}$H]sucrose ($1{\cdot}05 \pm 0{\cdot}05$, $16{\cdot}88 \pm 0{\cdot}77$ s, $0{\cdot}74 \pm 0{\cdot}10$, $n = 5$). The corresponding values for retrograde perfusions were: aspirin ($0{\cdot}73 \pm 0{\cdot}02$, $17{\cdot}41 \pm 3{\cdot}06$ s, $0{\cdot}32 \pm 0{\cdot}09$, $n = 5$), preformed [$^{14}$C]salicylic acid ($1{\cdot}14 \pm 0{\cdot}02$, $44{\cdot}42 \pm 3{\cdot}16$ s, $0{\cdot}95 \pm 0{\cdot}07$, $n = 5$), salicylic acid from aspirin ($0{\cdot}33 \pm 0{\cdot}09$, $36{\cdot}47 \pm 10{\cdot}28$ s, $0{\cdot}58 \pm 0{\cdot}05$, $n = 5$) and sucrose ($1{\cdot}01 \pm 0{\cdot}04$, $18{\cdot}08 \pm 1{\cdot}61$ s, $0{\cdot}76 \pm 0{\cdot}15$, $n = 5$).

No significant differences in F or MTT were apparent between anterograde and retrograde perfusions for all solutes. The MTT and $CV^2$ data for [$^{14}$C]salicylic acid and salicylic acid produced from aspirin is suggestive of a permeability limitation for salicylic acid transport.

While aspirin (acetylsalicylic acid) is a widely used drug, few studies have examined the disposition of aspirin and its major metabolite salicylic acid in the isolated perfused liver. A number of in-vivo and in-vitro studies on aspirin metabolism have quantified the hepatic availability (F) of aspirin where F is the fraction of aspirin escaping extraction by the liver on a single pass. The F values reported for aspirin range between 0·6 and 0·8 in man and dog (Harris & Riegelman 1969), sheep (Cossum et al 1986) and rat (Iwamoto et al 1982; Wientjes & Levy 1988). In contrast the F value for salicylic acid is close to unity (Cossum et al 1986; Ichikawa et at 1992; Shetty et al 1994).

In the present study, the outflow concentration–time profiles for aspirin and salicylic acid in the single pass in-situ perfused rat liver were examined after bolus input. The study sought to define the determinants of the mean transit time (MTT) of a sole metabolite produced from a precursor whilst varying the direction of flow through the liver. Some preliminary findings for aspirin and salicylic acid disposition in anterograde (normal) perfused isolated rat livers at different perfusion flow rates have been presented in abstract form elsewhere (Mellick & Roberts 1992). The present studies were conducted to define F and MTT for aspirin and its major metabolite salicylic acid using normal and retrograde perfusions. Normal vs retrograde perfusions are usually used to examine heterogeneous distributions of drug-metabolizing enzyme activity in the liver (Pang & Terrell 1981; Pang et al 1983; Xu et al 1990). In such studies, the relative proportions of metabolites are used to define the localization of metabolizing enzymes in the periportal and centrilobular regions of the liver (St-Pierre et al 1989).

In the present work, a mixture containing aspirin, a trace amount of radiolabelled [$^{14}$C]salicylic acid and an inert reference indicator [$^{3}$H]sucrose were injected as a bolus into a single-pass perfused rat liver and outflow fractions collected. Aspirin and salicylic acid produced from aspirin were analysed by HPLC whereas [$^{14}$C]salicylic acid and [$^{3}$H]sucrose were analysed by scintillation counting. F and MTT values for each outflow profile were then determined and analysed in terms of the flow conditions used. The study shows that a hepatocyte permeability barrier exists for salicylic acid and that there is no zonation in the metabolism of aspirin in the liver.

Correspondence: M. S. Roberts, Department of Medicine, The University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia.

## Materials and Methods

*Liver perfusion*

The in-situ perfused rat liver preparation has been described elsewhere (Roberts et al 1990a). Briefly, nine female Sprague–Dawley rats, 183–270 g (liver weights 5·4–10·4 g), having had free access to food and water, were anaesthetized by intraperitoneal injection of pentobarbital sodium (Nembutal, 80 mg kg$^{-1}$). Following laparotomy, the animals were given 100 μL heparin (100 int. units) via the inferior vena cava and the common bile duct was cannulated using PE 10 (polyethylene tubing, i.d. = 0·28 mm, o.d. = 0·61 mm, Clay Adams, New Jersey). The portal vein was then cannulated using an intravenous catheter placement unit (16 G i.d. = 1·30 × 32 mm, Terumo Medical Corporation, USA) and the liver was perfused with red blood cell-free and albumin-free Krebs-Henseleit bicarbonate buffer pH 7·4, which was pre-

equilibrated with carbogen gas (95% $O_2$/5% $CO_2$). The perfusion system was non-recirculating and employed a peristaltic pump (Cole-Palmer, Illinois) at a flow rate of 30 mL $min^{-1}$/liver. A silastic tubing lung ventilated with carbogen was used to maintain gaseous equilibrium. As soon as perfusion was effected, an incision was made in the inferior vena cava to release hepatic pressure and the thoracic inferior vena cava was cannulated with a 16 G intravenous catheter. Perfusions were always effected in the normal direction initially before the inflow was disconnected and flow was reversed with the liver being perfused via the thoracic inferior vena cava for retrograde flow. The flow reversal procedure typically took less than 5 s. In all experiments, the 16 G outlet catheter employed was connected to 9 cm of PE 240 tubing (i.d. = 1·67 mm, o.d. = 2·42 mm, length 10 cm, Clay Adams, New Jersey) from which the outflow samples were collected.

The perfusion preparation was carried out in a humidified cabinet and the temperature was maintained at 37 ± 2°C. Liver viability was assessed by macroscopic appearance, bile production, liver resistance and oxygen consumption. The time taken for the entire surgical procedure was typically 18–20 min. The study was approved by the University of Queensland Animal Experimentation and Ethics Committee.

*Impulse-response studies*

Bolus injections (50 μL) containing aspirin (approx. 200 μg) (Sigma, Sydney, Australia), [$^{14}$C]salicylic acid (1 μCi, approx. 3 μg) (Du Pont, Wilmington, DE, USA) and the extracellular marker [$^{3}$H]sucrose (5 μCi) (Du Pont, Wilmington, DE, USA), were injected via the inflow cannula. Following injection, outflow samples were collected in fractions over 2·5 min into plastic Eppendorf tubes via a fraction collector. In each liver, injections were made under conditions of normal and retrograde flow using a cross-over design. Aliquots of outflow samples (100 μL) containing [$^{14}$C]salicylic acid and [$^{3}$H]sucrose were counted in a Minaxi beta Tri-Carb 4000 series liquid scintillation counter (Packard Instruments Co., USA) to determine concentrations of radioactivity. Aspirin and its metabolite salicylic acid formed in the liver were assayed by HPLC as described below.

*Aspirin and salicylic acid HPLC assay*

Aspirin and its metabolite salicylic acid were assayed by HPLC using a modification of the methods of Owen et al (1987) and Rumble et al (1981). Perfusate samples (200 μL) were transferred to plastic Eppendorf tubes and 20 μL 35% perchloric acid was added followed by 400 μL acetonitrile containing internal standard (*p*-toluic acid, 0·004%) to precipitate any protein present. After the tubes were vortexed for 10 s and centrifuged (5 min at 9000 *g*), the clear supernatant (20 μL) was injected onto the column (5 micron Brownlee C18 reverse phase, Applied Biosystems, Foster City, CA, USA). The mobile phase consisted of 70 : 29 : 1 0·03% (v/v) phosphoric acid in water : acetonitrile : triethylamine at pH 2·0. The HPLC system consisted of a Waters 510 pump (flow rate 1 mL $min^{-1}$), model 710b automatic injector, model 480 UV detector (237 nm) and interface module (Waters, Millipore, Australia) with analysis of chromatograms performed using the Maxima 820 program (Waters, Millipore) on a personal computer.

Standard solutions ranging in concentration from 0·5 to 200 μg $mL^{-1}$ were prepared in buffer from 2 mg $mL^{-1}$ stock solutions of aspirin and salicylic acid. The aspirin and salicylic acid stock solutions were prepared by dissolving 0·01 g aspirin in 1 mL methanol and 4 mL buffer. The subsequent dilutions of this stock solution contained less than 1% methanol. All standards were prepared immediately before injection and were stored on ice. On occasion, salicylic acid standards were stored at 4°C and used again up to 48 h later.

The interday coefficients of variation for aspirin and salicylic acid were 9 and 12%, respectively (n = 5), while the within-day variability was found to be 3 and 1%, respectively (n = 5). The stability of an aspirin perfusate solution was determined at 4 and 25°C. The percentage of aspirin hydrolysed to salicylic acid over a 20-h period was 3 and 6% at 4 and 25°C, respectively. It was also found that on average 5% of the aspirin concentration in the injected sample was detected as salicylic acid. This breakdown contamination was accounted for in the moments analysis of data.

*Moments analysis*

The area under the solute concentration (C)–time (t) curves (AUC), the area under the first moment curve (AUMC) and normalized variance of the outflow concentration profiles ($CV^2$) were estimated using the parabolas-through-the-origin method (Purves 1992a, b, 1994).

The availabilities (F) for all solutes were then determined from the values of AUC of the solute using equation 1:

$$F = Q \cdot AUC/D \quad (1)$$

where Q is the perfusate flow rate and D is the dose of the solute administered.

The MTT was estimated by the ratio of AUMC to AUC:

$$MTT = AUMC/AUC \quad (2)$$

The normalized variance of the outflow concentration time profiles ($CV^2$) was determined according to equation 3:

$$CV^2 = \sigma^2/MTT^2 \quad (3)$$

where:

$$\sigma^2 = \frac{\int_0^\infty t^2 c(t)dt}{\int_0^\infty c(t)dt} - MTT^2 \quad (4)$$

The availability of metabolite produced from precursor was defined as:

$$F_{metabolite} = Q \cdot AUC_{metabolite}/D_{aspirin} \quad (5)$$

where $F_{metabolite}$ is the availability of salicylic acid produced from aspirin, $AUC_{metabolite}$ is the area under the curve for salicylic acid (expressed in aspirin equivalents) resulting from the administration of the dose of aspirin, $D_{aspirin}$.

*Statistics*

The moments of individual solutes and the effect of perfusion flow direction were compared using two-way repeated measures analysis of variance with the Newman–Keuls method used to identify groups which differed significantly. The number in each group of data was five and significance was taken at $P < 0·05$.

## Results

Fig. 1 shows typical normalized outflow concentration–time profiles for [$^3$H]sucrose, [$^{14}$C]salicylic acid, aspirin and salicylic acid produced from aspirin. The hepatic availability (F), mean transit time (MTT) and normalized variance ($CV^2$) deduced from the outflow profiles obtained are summarized in Table 1.

The experimental parameters associated with the normal vs retrograde perfusion studies (mean ± s.e., n = 5) were: rat weight 253 ± 4·4 g, liver weight 8·5 ± 0·2 g, bile flow 920 ± 140 $\mu$g min$^{-1}$ (g liver)$^{-1}$, liver resistance 6 ± 2 cm $H_2O$ and oxygen consumption 1·5 ± 0·1 $\mu$mol min$^{-1}$ (g liver)$^{-1}$. In each preparation, a homogeneous perfusion of the liver was confirmed from its macroscopic appearance.

*Hepatic availability*

Table 1 shows that aspirin is moderately extracted by the perfused rat liver under the conditions of this study; F = 0·73 ± 0·04 and 0·73 ± 0·02 for normal and retrograde perfusions, respectively. The remainder of the injected aspirin dose (33 ± 5% and 33 ± 9% for normal and retrograde perfusions, respectively) was recovered as salicylic acid. This suggests that salicylic acid does not undergo further metabolism in the perfused rat liver under the conditions employed. This is also apparent from the recovery of [$^{14}$C]salicylic acid which approaches unity (1·28 ± 0·04 (normal) and 1·14 ± 0·02 (retrograde)). A number of additional studies on [$^{14}$C]salicylic acid availability have been carried out and together with the present studies yielded an overall recovery of [$^{14}$C]salicylic acid close to unity (F = 1·05 ± 0·06, n = 12). The non-extracted reference compound [$^3$H]sucrose also had a recovery close to unity (F = 1·05 ± 0·05, n = 5). No significant differences in hepatic availability were observed between normal and retrograde perfusions for any of the solutes.

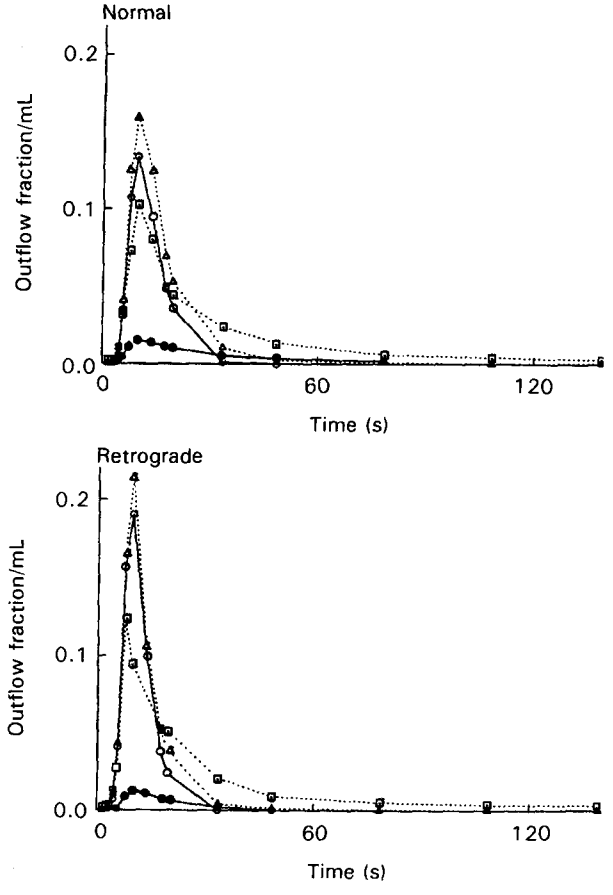


Fig. 1. Typical normalized concentration vs time profiles for aspirin (○), preformed salicylic acid ([$^{14}$C]salicylic acid) (▽), salicylic acid produced from aspirin in the liver (●) and [$^3$H]sucrose (□) under conditions of normal and retrograde flow in the in-situ rat liver perfused at a flow rate of 30 mL min$^{-1}$ with albumin and red blood cell-free Krebs buffer (pH 7·4). The normalized solute concentrations are expressed as the fraction of the total dose of solute per mL of collected perfusate. In the case of salicylic acid generated from aspirin, the concentration of salicylic acid (in aspirin equivalents) is expressed as the fraction of the aspirin dose per mL.

*Mean transit time (MTT)*

The MTT for aspirin in the liver is 15·13 ± 2·01 s under normal perfusion conditions. A not significantly different MTT is found under retrograde perfusion conditions (17·41 ± 3·06 s). These MTT values are significantly different from that of [$^{14}$C]salicylic acid (43·19 ± 2·21 s (normal) and 44·42 ± 3·16 s (retrograde)) and salicylic acid produced from aspirin (42·82 ± 9·16 s (normal) and 36·47 ± 10·28 s (retrograde)) ($P < 0·05$) but not significantly different from those of [$^3$H]sucrose (16·88 ± 0·77 s (normal) and 18·08 ± 1·61 s (retrograde)). Salicylic acid MTT values, whether given as preformed metabolite ([$^{14}$C]salicylic acid) or salicylic acid formed from aspirin, were similar and were more than twice that for parent aspirin and [$^3$H]sucrose. The values obtained are given in Table 1. No significant differences in MTT were observed between normal and retrograde perfusions for any of the solutes.

*Normalized variance ($CV^2$)*

In general the $CV^2$ values obtained from this study (Table 1) were within the range of those previously reported for other solutes (Roberts et al 1988; Evans et al 1993). The $CV^2$ value for aspirin was similar for normal (0·33 ± 0·09) and retrograde perfusions (0·32 ± 0·09). These values are generally less than half the values obtained for [$^3$H]sucrose, [$^{14}$C]salicylic acid and salicylic acid produced from aspirin (Table 1). No significant differences in $CV^2$ values were obtained for [$^3$H]sucrose, [$^{14}$C]salicylic acid and salicylic acid produced from aspirin. The $CV^2$ values for these solutes were similar for both normal and retrograde perfusions (Table 1).

## Discussion

*Hepatic availability*

The hepatic availability of aspirin determined by the present study (0·73) is similar in magnitude to that reported in in-vivo studies. Aspirin availabilities reported include 0·63 in dogs (Harris & Riegelman 1969), 0·67 in rats (Iwamoto et al 1982), 0·75 in sheep (Cossum et al 1986) and 0·8 in rats (Wientjes & Levy 1988). As the perfusate used in this study was albumin-free, the availability value of 0·73 appears somewhat higher than the predicted availabilities of 0·23 to 0·29 for in-vivo data assuming an aspirin fraction unbound of 0·36 (Cossum et al 1986). However, the perfusion flow rate used in this study (30 mL min$^{-1}$) is three times that normally observed in-vivo in order to ensure an adequate oxygen delivery (Gores et al 1986). Richardson & Witherington (1981) report flow rates of 10 to 15 mL min$^{-1}$ in the rat. Application of the well-stirred and

Table 1. Availability (F)·, mean transit time (MTT) and normalized variance ($CV^2$) data for aspirin·, preformed salicylic acid ([$^{14}$C]salicylic acid)·, salicylic acid produced from aspirin and [$^3$H]sucrose.

| Parameter | [$^3$H] Sucrose | | [$^{14}$C]Salicylic acid | | Aspirin | | Salicylic acid | |
|---|---|---|---|---|---|---|---|---|
| | Normal | Retrograde | Normal | Retrograde | Normal | Retrograde | Normal | Retrograde |
| Preparation | 9423 | | | | | | | |
| F | 1·07 | 1·07 | 1·37 | 1·07 | 0·86 | 0·65 | 0·39 | 0·53 |
| MTT | 17·72 | 20·35 | 46·97 | 49·94 | 15·21 | 18·50 | 74·68 | 74·82 |
| $CV^2$ | 0·63 | 0·48 | 0·99 | 0·82 | 0·25 | 0·35 | 0·91 | 0·67 |
| Preparation | 9424 | | | | | | | |
| F | 1·03 | 0·95 | 1·20 | 1·14 | 0·68 | 0·77 | 0·33 | 0·13 |
| MTT | 17·47 | 14·44 | 39·99 | 36·57 | 12·75 | 11·71 | 43·86 | 20·63 |
| $CV^2$ | 0·78 | 0·70 | 1·05 | 1·16 | 0·16 | 0·15 | 0·91 | 0·42 |
| Preparation | 9425 | | | | | | | |
| F | 1·22 | 1·12 | 1·26 | 1·19 | 0·66 | 0·72 | 0·19 | 0·10 |
| MTT | 18·79 | 17·38 | 39·60 | 38·96 | 13·48 | 11·65 | 24·26 | 18·25 |
| $CV^2$ | 0·82 | 1·30 | 1·34 | 1·08 | 0·18 | 0·12 | 0·38 | 0·49 |
| Preparation | 9426 | | | | | | | |
| F | 0·91 | 0·93 | 1·19 | 1·10 | 0·72 | 0·77 | 0·25 | 0·38 |
| MTT | 14·32 | 15·19 | 39·40 | 43·45 | 11·39 | 16·78 | 25·27 | 29·35 |
| $CV^2$ | 1·04 | 0·47 | 1·17 | 0·85 | 0·42 | 0·40 | 0·68 | 0·67 |
| Preparation | 9427 | | | | | | | |
| F | 1·00 | 0·98 | 1·37 | 1·18 | 0·74 | 0·74 | 0·50 | 0·49 |
| MTT | 16·12 | 23·03 | 49·99 | 53·16 | 22·82 | 28·39 | 46·05 | 39·28 |
| $CV^2$ | 0·42 | 0·58 | 0·84 | 0·82 | 0·63 | 0·60 | 0·75 | 0·67 |
| Mean ± s.e. | | | | | | | | |
| F | 1·05 ± 0·05 | 1·01 ± 0·04 | 1·28 ± 0·04 | 1·14 ± 0·02 | 0·73 ± 0·04 | 0·73 ± 0·02 | 0·33 ± 0·05 | 0·33 ± 0·09 |
| MTT | 16·88 ± 0·77 | 18·08 ± 1·61 | 43·19 ± 2·21 | 44·42 ± 3·16 | 15·13 ± 2·01 | 17·41 ± 3·06 | 42·82 ± 9·16 | 36·47 ± 10·28 |
| $CV^2$ | 0·74 ± 0·10 | 0·76 ± 0·15 | 1·08 ± 0·08 | 0·95 ± 0·07 | 0·33 ± 0·09 | 0·32 ± 0·09 | 0·73 ± 0·10 | 0·58 ± 0·05 |

tube models of hepatic elimination (Roberts & Rowland 1986) yield predicted availabilities of 0·35 and 0·5, respectively at 10 mL min$^{-1}$ using the observed availability of 0·73 at 30 mL min$^{-1}$ (Table 1). These predicted availabilities are similar in magnitude to the predicted unbound aspirin availabilities in-vivo. The recovery of [$^{14}$C]salicylic acid following bolus injection is effectively complete ($F = 1·05 \pm 0·06$, $n = 12$). No metabolites of salicylic acid were observed in any HPLC analyses of perfusate in which either aspirin or salicylic acid had been administered. Shetty et al (1994) recently showed that the extraction of salicylic acid in the perfused rat liver was negligible, the input and output concentrations of salicylic acid being identical in single pass steady-state infusion experiments. We were concerned that the work of Shetty et al (1994) was conducted at concentrations of salicylic acid exceeding the reported $K_m$ values for the salicylurate and salicylphenolic glucuronide pathways (Shen et al 1991). A repeat of Shetty's study at lower salicylic acid concentrations (including $^{14}$C-tracer concentrations of salicylic acid) showed no extraction of salicylic acid (unpublished results). However, when 20% red blood cells are added to the perfusate, availabilities for salicylic acid of about 0·8 can be estimated from the perfused rat liver data of Ichikawa et al (1992). The availability of salicylic acid in-vivo has been reported to be 0·91 in sheep (Cossum et al 1986). These workers also showed that the major metabolite of salicylic acid in the sheep (salicyluric acid) increases between the portal and hepatic veins after aspirin administration, demonstrating salicylic acid metabolism to salicyluric acid by the liver. This finding is in contrast with the assertion of Shetty et al (1994) that no metabolism of salicylic acid occurs in the liver.

*Mean transit time*

The MTT for aspirin (Table 1) was not significantly different from that of [$^3$H]sucrose, suggesting that these solutes occupy a similar volume of distribution in the liver. Sucrose occupies the extracellular spaces in the liver but does not enter the hepatocytes, while aspirin must at least enter the hepatocytes where elimination occurs. Roberts et al (1988) showed that according to the dispersion model, MTT is influenced by the volume into which a solute distributes and that an increase in extraction results in a decrease in MTT. Consequently aspirin, which has a moderate extraction (27%), has a similar MTT to that of the non-extracted sucrose. The MTT values for either preformed [$^{14}$C]salicylic acid or salicylic acid produced from aspirin in the liver are approximately twice that of aspirin and sucrose. The longer MTT for salicylic acid compared with aspirin and sucrose is most likely due to either differences in hepatocyte permeability for salicylic acid compared with aspirin, or to intracellular protein binding of salicylic acid. Salicylic acid has been shown to bind extensively to plasma proteins with approximately 2% unbound at low salicylic acid concentrations (Shen et al 1991). Aspirin binds less extensively with the unbound fraction in sheep plasma reported to be 36% (Cossum et al 1986). If we assume that the intracellular protein association constants for aspirin and salicylic acid are similar to those for plasma proteins, an increase in salicylic acid volume of distribution in the liver would be anticipated. The efflux permeability limitation for aspirin and salicylic acid would be expected to be comparable given their similar degree of ionization under the conditions used.

No significant differences in the MTT of any solute were observed between normal and retrograde perfusions. This sug-

gests that under the conditions of these experiments the apparent volumes into which the solutes distribute are independent of the direction of flow. Bolus studies using inert reference solutes have previously been used to examine the effects of retrograde flow on the vascular, Disse and intracellular water spaces in the liver (Bass et al 1989; Roberts et al 1990b; Xu et al 1990). These multiple indicator dilution studies have generally shown that the vascular space of the liver is slightly larger during retrograde perfusion. These findings have been attributed to differences in hepatic vascular resistance between normal and retrograde perfusion. The hepatic vascular resistance is controlled by sphincters at the inlet and outlet to the sinusoids. The sphincters which are usually inlet sphincters under conditions of normal flow become outlet sphincters under retrograde flow resulting in an increase in hepatic vascular resistance leading to an increased vascular space and thus increased MTT (Roberts et al 1990b). The interstitial, and cellular spaces are unaffected by the reversal of perfusion direction (Bass et al 1989; Roberts et al 1990b; Xu et al 1990). The independence of [$^3$H]sucrose MTT on the direction of flow in our present study indicates that the extracellular volume of the liver was unchanged on reversal of the direction of flow. The present work used a flow rate of 30 mL min$^{-1}$/liver and 16 G catheters at both the inlet and outlet. In contrast, previous studies have used lower flow rates — 10 mL min$^{-1}$/liver (Roberts et al 1990b), 8–12 mL min$^{-1}$/liver (Xu et al 1990) and 20 mL min$^{-1}$/liver (Bass et al 1989). It is possible, that as a consequence, the hepatic vascular resistance is dominated in this study by the resistance of the catheters rather than the outlet sphincters.

*Normalized variance ($CV^2$)*

The $CV^2$ data for [$^{14}$C]salicylic acid (Table 1) are also consistent with the possibility of an efflux limitation. Roberts et al (1990a) present expressions for $CV^2$ showing that $CV^2$ consists of two components: that due to vascular dispersion and that due to a hepatocyte permeability limitation. The vascular dispersion is a characteristic of the hepatic morphology alone (Evans et al 1993). The $CV^2$ value for [$^{14}$C]salicylic acid, which is approximately 30% greater than that for [$^3$H]sucrose, is therefore consistent with the presence of a permeability barrier. Ichikawa et al (1992) have previously suggested that salicylic acid is limited in its uptake by hepatocyte permeability. Roberts & Rowland (1986) also showed that an extracted solute will have a smaller $CV^2$ value than a reference solute. The $CV^2$ value for aspirin is less than half that for [$^3$H]sucrose consistent with the aspirin availability of 0·73.

## Conclusions

In this work, impulse–response studies have been used to determine hepatic availability, MTT and $CV^2$ for aspirin and salicylic acid in the in-situ perfused rat liver under conditions of both normal and retrograde flow. We revealed that aspirin is moderately extracted by the perfused rat liver ($F = 0{\cdot}73$) while the F value for salicylic acid is close to unity ($F = 1{\cdot}05$). The MTT values for both preformed and metabolite-generated salicylic acid were significantly longer than that for aspirin due to differences in intracellular protein binding between aspirin and salicylic acid. The hepatic availability and MTT for aspirin and salicylic acid were not influenced by reversing the direction of flow. $CV^2$ data were consistent with the existence of a permeability limitation for salicylic acid transport across the hepatocyte. These experimental results show the usefulness of bolus studies in the perfused rat liver for obtaining information regarding the disposition of both parent compounds and metabolites otherwise inaccessible using steady-state experiments.

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