lungs, respectively, values show mean of 2 measurements after 120 min perfusion).

Bakhle (1982) has shown that oedema induced in the rat in vivo causes a small reduction in the ex vivo pulmonary metabolism of $PGE_2$. However, there is no systematic study of the effects of oedema on the pulmonary inactivation of prostaglandins despite the fact that cyclo-oxygenase products may be important vasoactive mediators in primary pulmonary or non-cardiogenic oedema (reviewed by Brigham & Ogletree 1981).

We have shown elsewhere that the antigenic challenge elicits an anaphylactic response under the conditions used here (Robinson and Hoult 1980). In parallel experiments in this series we found that release into the effluent of immunoassayable 6-keto $PGF_{1\alpha}$, $PGE_2$ and $PGF_{2\alpha}$ was greater after antigen challenge in sensitized than in normal lungs ($1{\cdot}19 \pm 0{\cdot}25$ versus $0{\cdot}40 \pm 0{\cdot}07$, $P < 0{\cdot}01$; $0{\cdot}60 \pm 0{\cdot}09$ v. $0{\cdot}36 \pm 0{\cdot}05$, $P < 0{\cdot}05$ and $0{\cdot}27 \pm 0{\cdot}07$ v. $0{\cdot}15 \pm 0.04$, n.s., $n = 16$ from 4 lungs in each group, all values as ng $ml^{-1}$ in perfusates collected 20 to 480 s after challenge). Previous mass spectrometric analysis of anaphylactic guinea-pig lung perfusates has demonstrated these prostaglandins in the same order of abundance (Dawson et al 1976), but we were unable to assay immunologically for thromboxanes and prostaglandin metabolites which are present in larger amounts (Liebig et al 1974; Dawson et al 1976; Mathé et al 1977; Anhut et al 1978).

In summary, by studying the pulmonary inactivation of three prostaglandins we have failed to confirm the preliminary finding of others that this process is altered by sensitization or anaphylactic shock. Small time-dependent reductions in pulmonary degradation always occur in the absence of a colloid oncotic agent and may be related in part to the onset of oedema.

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# Rate limiting steps in metabolite kinetics: formation of 5-acetylaminosalicylate after administration of 5-aminosalicylate

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There are numerous examples of commonly prescribed drugs that have clinically important active metabolites (Drayer 1976; Atkinson & Strong 1977). In certain cases these active metabolites have been developed as drugs in their own right. The need to characterize the time course for an active metabolite in the body following administration of the parent drug has necessitated the development of certain pharmacokinetic models. We report information on the formation of the acetyl conjugate of 5-aminosalicylate after intravenous and oral administration of drug. The unusual behaviour apparent is discussed in relation to the more frequently encountered metabolite kinetic profiles.

* Correspondence.

Based on mass balance considerations, the rate of change of the amount of metabolite in the body is dependent upon both its formation rate and its elimination rate. Hence

$$V(m).\frac{dC(m)}{dt} = fm.CL.C - CL(m).C(m) \quad (1)$$

where the first term on the right hand side of this equation is concerned with metabolite formation. CL and C are the plasma clearance and concentration of parent drug, respectively, and fm is the fraction of the dose administered which is converted to the metabolite. The second term denotes metabolite elimination where CL(m) and C(m) are the plasma clearance and concentration of metabolite. V(m) is the metabolite volume of distribution.

When a drug is administered by a rapid intravenous bolus injection then its elimination from the body may be frequently described by

$$C = \frac{D}{V} \cdot e^{-k.t} \qquad (2)$$

where D is the dose administered in moles, V is the volume of distribution and k the overall elimination rate constant. Substitution of equation (2) into equation (1) remembering that CL = V.k, gives

$$V(m).\frac{dC(m)}{dt} = fm.k.D.e^{-k.t} - CL(m).C(m) \qquad (3)$$

Equation (3) may be integrated and rearranged to

$$C(m) = \frac{fm.k.D}{V(m).[k(m)-k]} \left(e^{-k.t} - e^{-k(m).t}\right) \qquad (4)$$

Thus, the rate limiting step governing the metabolite plasma concentration-time profile may be either the elimination rate constant of the drug or the elimination rate constant of the metabolite (Cummings et al 1967). In the first case the metabolite kinetics are *formation rate limited.* A semi-logarithmic plot of the metabolite data will show a linear terminal phase which reflects the half-life of the drug. Examples include the hydroxylated metabolite of tolbutamide (Matin & Rowland 1973) and naphthoxylactic acid, a metabolite of propranolol (Wälle et al 1979). Alternatively metabolite elimination may be the slowest step and the linear terminal phase of the semi-logarithmic plot reflects the metabolite elimination rate constant. Examples of *elimination rate limited* metabolite kinetics include the desmethyl metabolites of diazepam (Mandelli et al 1978) and aminopyrine (Lockwood & Houston 1980).

The metabolite plasma concentration-time profile will be dependent upon the route of administration of parent drug. When the drug is administered orally, provided absorption is complete, the time course of drug plasma concentrations may be described as

$$C = \frac{ka.D}{V(ka-k)} \left(e^{k.t} - e^{-ka.t}\right) \qquad (5)$$

where ka is the absorption rate constant. Provided that first pass metabolite production is minimal, equation (5) my be substituted into equation (1) and the resulting equation integrated. Hence equation (6) describing metabolite plasma concentrations following oral drug administration is analogous to equation (4) for the intravenous route.

$$C(m) = \frac{fm.k.ka.D}{V(m)} \left(\frac{e^{-k.t}}{[k(m)-k]\,[ka-k]} + \frac{e^{-k(m).t}}{[k(m)-k]\,[k(m)-ka]} - \frac{e^{-ka.t}}{[k(m)-ka]\,[ka-k]}\right) \qquad (6)$$

Although equation (6) is triexponential it is unlikely that the three rate constants will differ sufficiently to be resolved from a semi-logarithmic plot by curve stripping. However, as pointed out by Rowland & Tozer (1980) there is a third potential rate-limiting case for metabolite kinetics when the drug is given by the oral route. If the drug kinetics are governed by absorption (that is, ka < k in equation (5): the 'flip-flop' situation as discussed by Gibaldi & Perrier (1975) and Byron & Notari (1976) and the elimination of metabolite is rapid then *absorption rate limited* metabolite kinetics may occur. In other words the smallest exponent in equation (6) is ka. To date there would appear to be no documented examples of this phenomenon. The data described below are compatable with both 5-aminosalicylate and its metabolite 5-acetylaminosalicylate displaying absorption rate limited kinetics.

Male Sprague-Dawley rats, mean weight 340 g, were anaesthetized with urethane and surgically prepared for collection of blood samples and for administration of drug by the intra-arterial and intraduodenal routes as previously described (Cassidy & Houston 1980). 5-Aminosalicylate (5 mg $kg^{-1}$) was administered in aqueous solution to 10 animals (5 rats per route) and serial blood samples (100 µl) removed over 160 min.

Plasma was mixed with an equal volume of methanol containing salicylate (internal standard) and centrifuged. The supernatant was assayed for aminosalicylate and acetylaminosalicylate by high performance liquid chromatography. A Waters M6000A pump with a Schoeffel F8970 fluorescence detector (excitation 310 nm and emission cut off filter 385 nm) was used. A 200 × 5 mm (i.d.) stainless steel column packed with Hypersil 5-SAS (Shandon Southern Ltd) was preceded by a 50 × 5 mm (i.d.) stainless steel pre-column packed with silica. The mobile phase consisted of 30% methanol in 0·1 M phosphate buffer pH 7·4 containing 0·15% tetrabutylammonium.

Following intra-arterial administration the plasma concentration of aminosalicylate decline in a biexponential fashion (see Fig. 1) with an initial half-life of 4 min (s.d. 2) and a terminal half-life of 17 min (s.d. 4). The acetyl conjugate plasma concentrations peaked rapidly and declined with a half-life of 53 min (s.d. 4). A paired *t*-test demonstrated that the metabolite half-life was statistically significantly different ($P < 0{\cdot}005$) from the drug half-life indicating elimination rate limited metabolite kinetics following intra-arterial administration of drug. Urinary excretion experiments showed complete recovery of the dose administered in the form of the acetyl conjugate. Plasma clearances for aminosalicylate and acetylaminosalicylate calculated by the area under curve method were 25·3 ml $min^{-1}$ $kg^{-1}$ (s.d. 6) and 8·5 ml $min^{-1}$ $kg^{-1}$ (s.d. 3), respectively.

Plasma concentration-time profiles for both aminosalicylate and its metabolite are drastically altered when the drug is administered orally (see Fig. 1). Table 1 shows that the half-life for both compounds and the time taken to attain maximum metabolite concentration is increased significantly when compared with the intra-arterial route. There is no statistical difference between

the drug and metabolite half-lives after oral administration (by paired $t$-test). These observations suggest that the kinetics of aminosalicylate and acetylaminosalicylate are absorption-rate limited following intraduodenal dosing.

The area under the plasma concentration-time curve for both drug and metabolite is lower following intraduodenal administration than when the intra-arterial route is used (Table 1). There is also a decrease in the urinary recovery of acetyl conjugate (66% of dose, s.d. 9) but no statistical change in the plasma clearance of this metabolite (9·2 ml $min^{-1}$ $kg^{-1}$, s.d. 4). No unchanged drug was found in the urine. Treatment of urine with either β-glucuronidase (Sigma, Type H1), or 2 M hydrochloric acid did not increase recovery of drug and this would suggest that there is no route-dependent conjugation of aminosalicylate with glucuronic acid or glycine. The above findings together with the slow rate of absorption observed strongly indicate incomplete absorption of this drug from the intestinal tract.

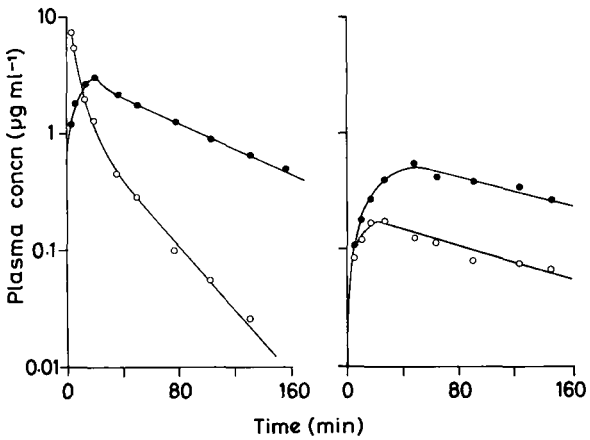


FIG. 1. Plasma concentration-time profile for aminosalicylate (○) and acetylaminosalicylate (●) after administration of aminosalicylate to a rat by the intra-arterial (left panel) and intraduodenal (right panel) route.

Table 1. Influence of route of administration on the pharmacokinetics of 5-aminosalicylate and its metabolite acetylaminosalicylate.

| Parameter | Route of administration[a] Intra-arterial[a] | Intraduodenal[a] | Statistical difference between routes[b] |
|---|---|---|---|
| Drug AUC[c] (μg $ml^{-1}$ min) | 210 (50) | 93 (45) | $P < 0.01$ |
| Drug terminal half-life (min) | 17 (4) | 76 (24) | $P < 0.001$ |
| Metabolite AUC[c] (μg $ml^{-1}$ min) | 476 (220) | 279 (130) | NS |
| Metabolite terminal half-life (min) | 53 (3) | 88 (26) | $P < 0.05$ |
| Time of maximum metabolite concn (min) | 10 (4) | 58 (15) | $P < 0.001$ |

[a] Mean of 5 rats with standard deviation.
[b] By $t$-test.
[c] AUC, area under the plasma concentration-time curve.

5-Aminosalicylate is thought to be the active moiety of the drug sulphasalazine which is widely used for inflammatory bowel disease (Klotz et al 1980). A small fraction of the sulphasalazine dose administered is absorbed from the small intestine but most reaches the colon intact where it undergoes azo-reduction to liberate aminosalicylate and sulphapyridine (Das & Dubin 1976). Thus, sulphasalazine would appear to act as a vehicle to achieve high concentrations of aminosalicylate in the intestinal tract. Despite the high doses administered (2–4 g daily), plasma concentrations of aminosalicylate and acetylaminosalicylate following chronic sulphasalazine administrations are low (Schroder & Campbell 1972) and very constant within a dosing interval (Fischer & Klotz 1979; Sivner, Martin, Shaffer & Houston, unpublished observations). The results reported herewith would suggest that this is a consequence of slow absorption rather than disposition of aminosalicylate and its acetyl conjugate.

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