Biochemical Pharmacology, Vol. 31, No. 16, pp. 2649–2656, 1982.
Printed in Great Britain.

0006-2952/82/162649-08 $03.00/0

THE ROLE OF THE GUT FLORA IN THE REDUCTION OF SULPHINPYRAZONE IN THE RAT

ANDREW G. RENWICK,* SUSAN P. EVANS, TREVOR W. SWEATMAN, JANET CUMBERLAND and CHARLES F. GEORGE

Clinical Pharmacology Group, University of Southampton, Medical and Biological Sciences Building, Bassett Crescent East, Southampton SO9 3TU, U.K.

(*Received* 6 *January* 1982; *accepted* 16 *March* 1982)

Abstract—Sulphinpyrazone underwent both reduction to a sulphide and oxidation to a sulphone after parenteral administration to normal Wistar rats. Oral administration was associated with a bioavailability of about 75% and with a 3-fold greater formation of the sulphide. However, no sulphide was detected in the plasma after oral administration of sulphinpyrazone to germ-free (BD/X) rats or normal rats treated with oral antibiotics. *In vitro* studies showed that the major site of reduction of sulphinpyrazone was the contents of the hind gut with little activity detected in the liver or other tissues. The sulphide was oxidised *in vivo* to sulphinpyrazone and small amounts of sulphone, while the latter underwent only slight reduction to sulphinpyrazone, but did not give detectable levels of the sulphide. These data suggest that the gut microflora are the main site of reduction of sulphinpyrazone in the rat *in vivo*.

Sulphinpyrazone [1,2-diphenyl-3,5-dioxo-4-(2'-phenylsulphinylmethyl) pyrazolidine; Anturan; see Fig. 1] which has been used for many years for the treatment of gout, has recently received renewed interest due to its platelet anti-aggregatory activity [1] and its potential therapeutic role in the prevention of reinfarction following myocardial infarction [2, 3]. Recent studies have shown that sulphinpyrazone is converted in man and other species to a sulphide metabolite [4] which is about 12 times more potent than the parent compound at inhibiting arachidonate induced platelet aggregation. In man the sulphide has a longer half-life than the parent compound and represents a major circulating metabolite during chronic administration [5].

The site of the reduction *in vivo* had not been investigated and may be either the liver, as has been suggested for the reduction of the SO group of the drug sulindac [6], or the intestinal flora as has been demonstrated for the reduction of nitro [7], *N*-hydroxy [8], *N*-oxide [9] and azo compounds [10], and also arsonic acids [11]. This paper reports on the site of reduction of sulphinpyrazone in the rat.

* Author to whom correspondence should be addressed.

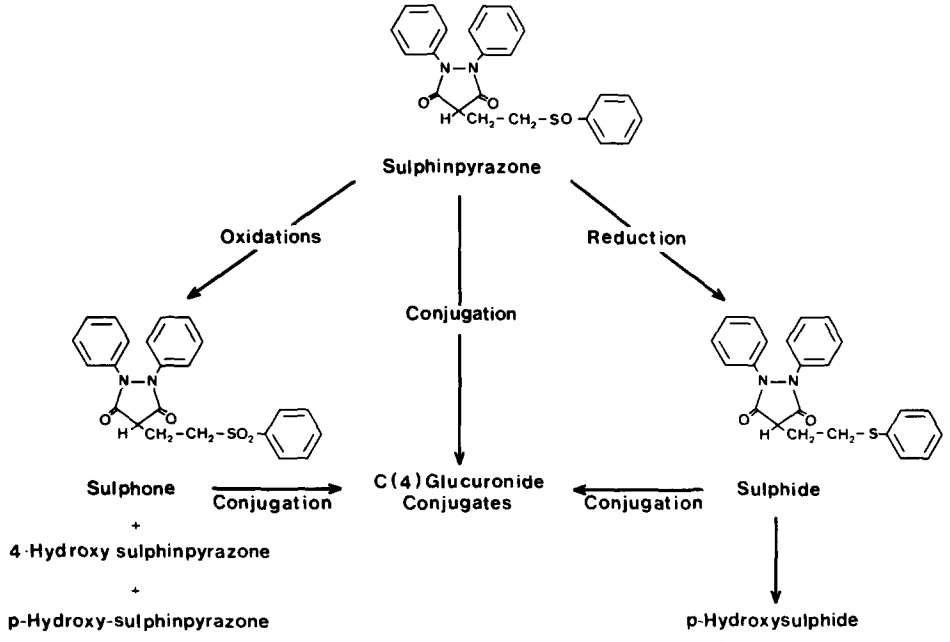

Fig. 1. The metabolic fate of sulphinpyrazone in man and animal species.

MATERIALS AND METHODS

Sulphinpyrazone and its sulphide, *p*-hydroxysulphide, sulphone and *p*-hydroxy- metabolites were gifts from Geigy Pharmaceuticals (Horsham, U.K.) and fenbufen was kindly supplied by Lederle Laboratories (Gosport, U.K.). All chemicals were Analar or HPLC grade from BDH (Poole, U.K.) or Rathburn Chemicals (Walkerburn, U.K.).

Determination of sulphinpyrazone and its metabolites. Sulphinpyrazone and its metabolites in plasma and incubates were analysed by HPLC. The internal standard (fenbufen; 5.0 μg in 50 μl methanol) was added to each plasma sample which was then acidified by the addition of 0.1 M HCl (2.0 ml) and extracted with chlorobutane:1,2-dichloroethane (4:1 v/v; 5.0 ml). The upper organic layer was separated by centrifugation, removed and shaken with 0.1 M NaOH (400 μl) to extract the acidic drug and metabolites. The alkali was separated by centrifugation and an aliquot (up to 200 μl) injected into an HPLC system comprising a Waters M6000A pump, U6K injector and μBondapak C_{18} column (30 cm; 3.9 mm i.d.). The mobile phase was 0.05 M aq. $NH_4H_2PO_4$ (pH 5.9); acetonitrile (71:29 v/v) at a flow of 2.0 ml/min and peaks were detected by u.v. absorption at 254 nm using a Waters Model 450 or Model 440 detector (see Fig. 2). The peak areas were measured using an Infotronics Model 308 integrator and the concentration determined by comparison with known standards extracted and analysed with each batch of samples. *p*-Hydroxysulphinpyrazone eluted before the sulphone and was not determined due to the presence of interfering peaks. The *p*-hydroxysulphide, which decomposed slowly in alkaline solution, eluted between the sulphone and internal standard, but was not detected in any of the samples analysed.

In vivo *experiments.* Male Wistar albino rats (body wt 300 ± 50 g), bred from a colony maintained at the Medical and Biological Sciences Building, were used. The animals were given free access to food and water prior to and throughout each study except where indicated. Male germ-free rats (BD/X; body wt 170–310 g) were obtained from the MRC Laboratory Animal Centre (Carshalton, U.K.) and were kept in a room separate from other animals and given sterilised food and water *ad libitum*. The germ-free rats were dosed within 1 hr of opening the sterile transport container. Male Wistar rats treated with antibiotics were given the low dose regimen of neomycin, bacitracin and tetracycline as described previously [7]. In some animals (Table 1) the bile duct was ligated under pentobarbitone anaesthesia, prior to dosing and subsequently throughout the study.

Sulphinpyrazone was administered by various routes (Table 1) as a 10 mg/ml solution prepared in water or 0.9% saline by the addition of a slight excess of $NaHCO_3$. The sulphide and sulphone were administered by various routes (Table 2) as 10 mg/ml solution prepared by dissolution in 0.1 M NaOH followed by the addition of sodium bicarbonate to adjust the pH to about 9. Intravenous injections were performed via the external jugular vein under pentobarbitone anaesthesia from which the animals

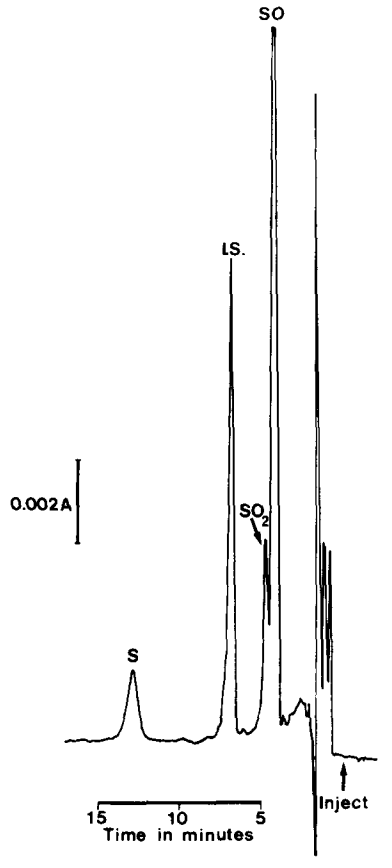

Fig. 2. HPLC analysis of sulphinpyrazone and its metabolites. The trace shown is that of a plasma sample collected 6 hr after an oral dose of sulphinpyrazone given to a normal rat. Details of the extraction procedure and HPLC conditions are given in the text. SO, sulphinpyrazone; SO_2, sulphone metabolite; I.S., internal standard; S, sulphide metabolite.

recovered within 2–3 hr. Blood samples (0.3 ml; up to 10 per animal) were removed by cardiac puncture under temporary ether anaesthesia, at intervals from 15 min up to 96 hr after dosing, during which time the animals ate and drank normally. Autopsy revealed negligible cardiac damage or haemorrhage, and the haematocrit remained normal for all animals reported in this paper.

Attempts to measure the urinary excretion of the sulphide or its hydroxylated conjugated metabolite, as an indication of the extent of reduction *in vivo*, proved impracticable due to the very low levels present. Thus, the extent of formation of metabolites was assessed from plasma data by measurement of the area under the plasma concentration–time curve (AUC) by the trapezoid rule.

In vitro *experiments. Tissue incubations.* The tissues were removed and prepared as 33% w/v homogenates in 0.1 M phosphate buffer pH 7.4, under an atmosphere of oxygen free nitrogen. The homogenates (1 ml) were incubated with substrate (50 μg sulphinpyrazone or 5 μg sulphide metabolite in 1 ml phosphate buffer, pH 7.4) for 1 hr at 37° by shaking under an atmosphere of oxygen free nitrogen and

Table 1. Plasma pharmacokinetic data for sulphinpyrazone in the rat

Route*	Dose (mg/kg)	Animal type†	Number	SO Peak concn (μg/ml)	SO AUC (μg/ml·hr)	SO $t_{\frac{1}{2}}$ (hr)	S Peak concn (μg/ml)	S AUC (μg/ml·hr)	S $t_{\frac{1}{2}}$ (hr)	SO_2 Peak concn (μg/ml)	SO_2 AUC (μg/ml·hr)	SO_2 $t_{\frac{1}{2}}$ (hr)
i.v.	20	N	4	—	1197 (284)	12.3 (1.7)	2.1 (0.5)	61 (16)	16.9 (2.9)	4.0 (0.1)	29 (5)	6.8 (0.7)
i.p.‡	20	N	11	—	1263 (221)	13.8 (2.9)	1.6 (0.6)	52 (14)	17.9 (8.1)	5.4 (1.0)[6a]	23 (6)[6]	2.3 (0.5)[6c]
Oral (fed)§	20	N	7	60 (16)	1033 (166)[a]	15.8 (6.3)	5.5 (1.7)[c]	146 (59)[c]	13.0 (6.5)	2.4 (0.8)	23 (7)	8.6 (4.2)
Oral (fasted)‖	20	N	8	62 (9)	817 (159)[a]	11.1 (2.2)	8.7 (5.3)	153 (70)	9.6 (6.3)	4.8 (2.8)[4a]	24 (10)[4]	2.4 (0.4)[4a]
Oral (fed)‖	20	GF	6	50 (29)	496 (121)[c]	9.9 (0.9)[a]	ND[c]	ND[c]	—	0.7 (0.2)[c]	4 (2)[c]	5.2 (3.0)
Oral (fed)‖	20	AB	6	84 (48)	1190 (528)	20.7 (11.4)	ND[c]	ND[c]	—	TR	—	—
i.v.‡	20	BL	3	—	2581 (1450)	46.9 (20.4)	1.4 (0.3)	120 (71)	60.7 (57.4)	6.5 (1.5)[a]	96 (10)[c]	28.4 (11.6)[a]

The results are the means with standard deviations in parentheses for all animals in each treatment group except where indicated by a superscript.

* Compounds were given by intragastric intubation (oral) or by intravenous (i.v.) or intraperitoneal (i.p.) injection.

† N, normal; GF, germ-free; AB, antibiotic treated; BL, bile ligated.

AUC, area under plasma concentration–time curve from time zero to infinity; ND, not detected; TR, trace level present but insufficient to define pharmacokinetic parameters.

SO, sulphinpyrazone; S, sulphide metabolite; SO_2, sulphone metabolite.

‡ Results compared with normal rats given an i.v. dose: [a] $P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

§ Results compared with normal rats given an i.p. dose.

‖ Results compared with fed normal rats given an oral dose.

Table 2. Plasma pharmacokinetic data for the sulphide and sulphone

	Route	Dose (mg/kg)	Animal number	SO: Peak concn (μg/ml)	SO: AUC (μg/ml·hr)	SO: $t_{\frac{1}{2}}$ (hr)	S: Peak concn (μg/ml)	S: AUC (μg/ml·hr)	S: $t_{\frac{1}{2}}$ (hr)	SO_2: Peak concn (μg/ml)	SO_2: AUC (μg/ml·hr)	SO_2: $t_{\frac{1}{2}}$ (hr)
Sulphide												
	i.v.	20	4	17.4 (1.9)	507 (49)	13.1 (1.5)	—	1237 (149)	6.9 (1.3)	TR	—	—
	s.c.	20	4	19.1 (2.0)	677 (42)	16.5 (0.5)	137.3 (19.2)	1655 (261)	8.9 (0.4)	0.6 (0.4)[3]	12 (9)	11.7 (2.5)
	s.c.	5	6	2.9 (0.5)	114 (18)	15.8 (2.6)	33.8 (7.1)	374 (68)	7.9 (1.0)	TR	—	—
	Oral	5	5	3.0 (0.8)	120 (22)	20.3 (10.7)	6.1 (7.1)	292 (25)	11.5 (2.9)	TR	—	—
	Oral	2	4	1.2 (0.1)	53 (15)	15.2 (2.8)	5.4 (1.1)	115 (12)	9.5 (1.7)	ND	ND	—
Sulphone												
	i.v.	5	4	1.6 (0.1)	7 (3)	10.8 (8.8)	ND	ND	—	—	36 (7)	4.0 (3.0)
	s.c.	10	6	2.8 (0.6)	38 (8)	17.5 (3.9)	ND	ND	—	28.7 (6.7)	86 (13)	2.8 (0.5)
	Oral	20	4	1.2 (0.2)	21 (2)	12.1 (3.1)	ND	ND	—	13.8 (4.8)	96 (10)	5.1 (2.0)

The results given are the mean values for normal rats with the standard deviations in parentheses (for all animals except where indicated by a superscript). s.c., subcutaneous; all other abbreviations are as given in Table 1.

the reaction stopped by the addition of fenbufen (25 μg in 25 μl methanol) and 2 M HCl (2.0 ml). The incubates were then extracted with chlorobutane:1,2-dichloroethane and analysed by HPLC as described above.

Faecal incubations. A fresh faecal pellet was added to a pre-weighed tube containing 0.1 M phosphate buffer pH 7.4 (1.0 ml). Substrate (50 μg sulphinpyrazone in 1 ml phosphate buffer pH 7.4) was added, and the tube incubated as described above and analysed for metabolites by HPLC.

RESULTS

Sulphinpyrazone

After intravenous administration of sulphinpyrazone (20 mg/kg) to anaesthetised, normal rats there was a slow decrease in plasma concentrations of parent compound ($t_{\frac{1}{2}}$ = 12 hr) (Table 1). The plasma concentration of the sulphide metabolite showed a peak (2 μg/ml) about 10 hr after the dose and decreased slowly thereafter ($t_{\frac{1}{2}}$ = 17 hr). The sulphone metabolite reached a peak plasma concentration (4 μg/ml), about 2 hr after the dose and decreased more rapidly ($t_{\frac{1}{2}}$ = 7 hr). Comparison of the AUC values for the sulphide (61 μg/ml·hr) and sulphone (29 μg/ml·hr) with those obtained after parenteral injection of these metabolites (Table 2) (sulphide—average AUC/mg/kg, 73.2 μg/ml·hr; sulphone—average AUC/mg/kg, 7.9 μg/ml·hr) suggests the formation of 0.8 mg/kg of sulphide (4.3% of the dose) and 3.7 mg/kg of sulphone (18% of the dose) after intravenous injection of sulphinpyrazone (20 mg/kg). Almost identical results (3.7% sulphide; 14% sulphone) were obtained following intraperitoneal injection of sulphinpyrazone to a larger group of animals.

After oral administration of sulphinpyrazone to normal rats the AUC for the parent compound was reduced by 16% in fed and 34% in fasted animals compared with the mean parenteral value, suggesting incomplete absorption from the gut and/or first-pass metabolism (Table 1). The difference in AUC between the fed and fasted rats arose from the difference in half-life in the two groups rather than the maximum plasma concentration detected. The peak plasma concentrations of the sulphide metabolite, which occurred about 12 hr after dosing in fed and fasted rats (5.5 and 8.7 μg/ml respectively) were significantly higher than following parenteral administration, and this was accompanied by significantly higher AUC values, despite the shorter half-life of the sulphide detected in these animals. The pharmacokinetic data for the sulphone metabolite were not affected by the route of administration.

After oral administration of sulphinpyrazone to germ-free rats (Table 1) the peak plasma concentrations, AUC and half-life of the parent compound were reduced compared with normal rats. However, while small amounts of the sulphone were present, no sulphide metabolite was detected in the plasma of these rats. Antibiotic treatment of normal rats also prevented formation of the sulphide, but in this case the data for the parent compound were unaltered. The concentration of the sulphone in plasma was reduced by antibiotics but was not quantified, due partly to poor HPLC separation from the parent compound in these samples.

Intravenous administration of sulphinpyrazone to animals in which the bile duct was ligated (Table 1) showed that the half-life and AUC values for the parent drug and both metabolites were increased, indicating the importance of biliary excretion in the overall fate of sulphinpyrazone in the rat.

Sulphide derivative

Intravenous and subcutaneous administration of the sulphide metabolite (Table 2) was followed by a more rapid decrease in plasma concentrations than was detected when this compound was formed after sulphinpyrazone administration. The sulphide was metabolised to sulphinpyrazone *in vivo* and comparison of AUC values (Table 2) with those obtained after parenteral administration of sulphinpyrazone (Table 1) suggests the formation of 8.2 mg/kg (39% of the dose) after intravenous dosing (20 mg/kg). Similar results were obtained after subcutaneous administration of 20 mg/kg and 5 mg/kg, i.e. 11.0 mg/kg (55% of dose) and 1.85 mg/kg (37% of dose) respectively as sulphinpyrazone. After oral administration of the sulphide (5 mg/kg or 2 mg/kg) the peak plasma level and AUC of the sulphide were lower than after subcutaneous dosing suggesting a bioavailability from the gut of about 80%. Again, sulphinpyrazone was detected, equivalent to 1.95 mg/kg (37% of the dose) and 0.86 mg/kg (41% of the dose) after 5 mg/kg and 2 mg/kg doses, respectively. The sulphone was detected in the plasma at very low levels and was quantifiable in 3 out of 4 animals after subcutaneous injection of 20 mg/kg of sulphide. Assuming that the fourth rat formed an amount equal to the limit of detection, the average amount of sulphone formed was equivalent to an AUC of 12 μg/ml·hr or 1.5 mg/kg of sulphone, i.e. 7% of the dose. This is similar to the value (9% of dose) expected from the amount of sulphinpyrazone formed (55%) in these animals.

Sulphone derivative

Comparison of the AUC values after i.v. and oral doses of the sulphone (Table 2) suggested a bioavailability of about 60% after oral administration with about 0.1 mg/kg (2% of the dose), 0.6 mg/kg (6% of the dose) and 0.3 mg/kg (2% of the dose) converted to sulphinpyrazone after intravenous (5 mg/kg), subcutaneous (10 mg/kg) and oral (20 mg/kg) doses. No sulphide was detected in the plasma of these animals.

Anaerobic incubations

Anaerobic incubation of the tissues of normal rats with sulphinpyrazone (Table 3a) showed that the major site of reduction to the sulphide was the contents of the caecum. The liver and the contents of the small intestine showed a slight ability to form the sulphide but were 100 times less active than the caecal contents. Under the anaerobic conditions employed, little sulphide was converted to sulphinpyrazone (Table 3b) and the recovery of sulphide ranged from 26–94%. The distribution of the sulphinpyrazone reducing activity along the contents of the gastrointestinal tract is given in Fig. 3. Incu-

Table 3. The metabolism of sulphinpyrazone *in vitro*

(a) Sulphinpyrazone (50 μg) incubations

Tissue	N	μg recovered as Sulphinpyrazone	μg recovered as Sulphide
Liver	5	55.4 (4.7)	0.22 (0.13)
Kidney	5	50.3 (5.3)	0.02 (0.03)
Spleen	5	45.1 (4.1)	0.06 (0.04)
Small intestine			
Wall	5	46.9 (3.7)	0.04 (0.05)
Contents	5	42.6 (3.8)	0.37 (0.27)
Caecum contents	5	4.5 (4.1)	36.24 (5.06)
Boiled caecum contents	3	39.9 (2.5)	0.01 (0.01)

(b) Sulphide (5 μg) incubations

Tissues	N	μg recovered as Sulphinpyrazone	μg recovered as Sulphide
Liver	3	0.20 (0.09)	2.68 (1.00)
Kidney	3	0.23 (0.13)	3.05 (0.35)
Spleen	3	0.23 (0.13)	2.12 (0.17)
Small intestine			
Wall	3	0.30 (0.50)	1.32 (0.49)
Contents	3	0.23 (0.27)	3.16 (1.00)
Caecum contents	3	0.06 (0.06)	4.74 (1.52)

Tissues were prepared as 33% w/v homogenates in 0.1 M phosphate buffer pH 7.4 and an aliquot (1 ml) incubated with substrate (1 ml) at 37° for 1 hr under oxygen-free nitrogen. The results given are the mean with the standard deviation in parentheses.

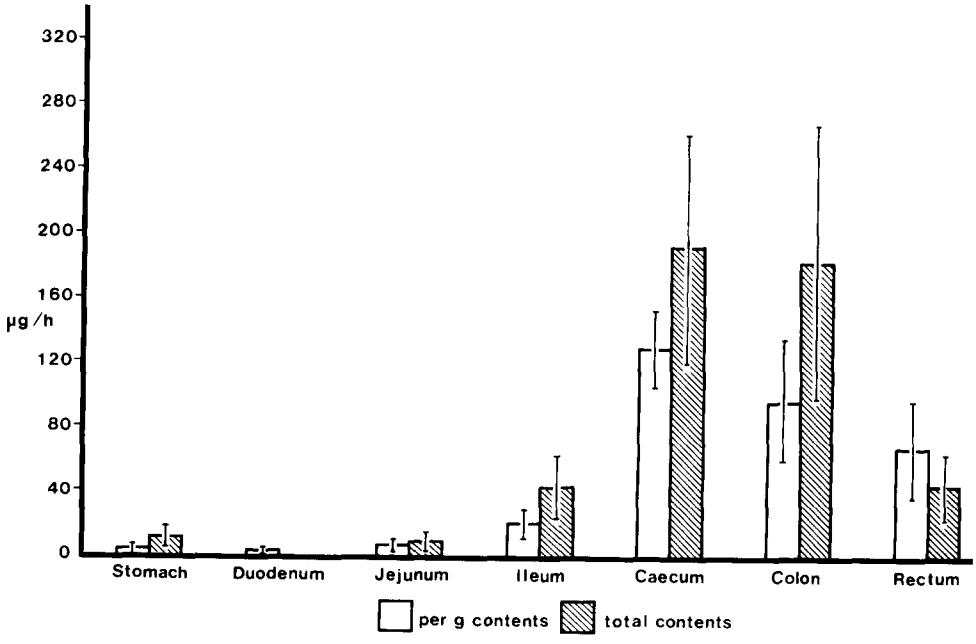

Fig. 3. The distribution of sulphinpyrazone reducing activity in the gastrointestinal contents of the rat. The gut contents were prepared as 33% homogenates and incubated with sulphinpyrazone (50 μg) for 1 hr. The results given are the formation of the sulphide metabolite in μg per gram of contents and per total contents. The results are the mean of 3 animals with the standard error indicated by the vertical line.

bation of faecal pellets from germ-free rats during the 5 days of the *in vivo* study showed no detectable reduction of sulphinpyrazone to the sulphide whereas faeces from normal rats reduced 55 ± 10% of the sulphinpyrazone added (50 μg) under similar conditions.

DISCUSSION

Comparison of the fate of sulphinpyrazone after oral and parenteral administration showed a route of administration difference in the extent of reduction to the sulphide. About 3 times as much sulphide was formed after oral as after parenteral dosing (Table 1), suggesting an extensive role for the gastrointestinal tract in this reaction.

The *in vitro* incubation results (Table 3) showed that the major site of reduction was the contents of the caecum, with negligible activity associated with other sites including the wall of the small intestine and the liver. The distribution of sulphinpyrazone reducing activity along the gastrointestinal tract contents (Fig. 3) showed that the major site of metabolism was the hind gut and the distribution obtained was typical of that seen for other reactions performed extensively by the gut microflora [12]. The 12 hr delay between oral administration of sulphinpyrazone and the appearance of the peak plasma concentration of the sulphide was probably due to the time taken for the dose to pass down the gut, to the site of reduction, which is about 4 hr in the rat [13], combined with a slow formation by the gut flora.

In vivo studies on the fate of an oral dose of sulphinpyrazone in germ-free and antibiotic treated rats showed that no sulphide was produced in rats in which the gut microflora were absent or suppressed. Similarly, no sulphide was formed *in vitro* on incubation of faeces from germ-free rats with sulphinpyrazone. These results, therefore, suggest that *in vivo* the gut micro-organsims are the major, if not the sole, site of conversion of sulphinpyrazone to its active sulphide metabolite in the rat.

The extent of sulphide formation after intravenous administration of sulphinpyrazone was about one-half that found by Dieterle and Faigle [14] using [^{14}C]sulphinpyrazone (100 mg/kg) and reverse isotope dilution, based on measurements of total ^{14}C 0–24 hr AUC and ^{14}C sulphide AUC. However, their results are not strictly comparable with ours because the AUC for ^{14}C sulphide was not related to a known systemic dose of the metabolite. Furthermore, the plasma concentration–time curve needs to be followed for some 72 hr to allow adequate measurement of the AUC (to infinity) for both parent compound and the sulphide.

The presence of the sulphide metabolite in the plasma after parenteral dosing with sulphinpyrazone indicates that the latter enters the gut, probably via the bile. The elimination half-life of the sulphide was considerably greater when produced as a metabolite of sulphinpyrazone (Table 1), than when given by parenteral injection (Table 2), which is consistent with the presence of an entero-hepatic circulation of sulphinpyrazone and subsequent formation of the sulphide metabolite by the gut flora. However, the molecular weight of sulphinpyrazone (404) is such that there may be marked species differences in the extent of biliary excretion [15] and as a result, in the amount of sulphide formed. A marked inter-species variation in the excretion of ^{14}C in the faeces was found after an intravenous dose of [^{14}C]sulphinpyrazone (100 mg/kg) [14], with the rat showing the highest faecal excretion (73% of the dose). In view of the high faecal elimination, the 3-fold route of administration difference found for sulphide formation in the present study appears greater than might be expected. This suggests that either the biliary material is not completely converted to the sulphide, or that there is a significant dose-dependency in the route of excretion, with decreased biliary elimination at 20 mg/kg as used in this study compared with 100 mg/kg. In addition, some faecal excretion of sulphinpyrazone may reflect elimination across the wall of the intestine, since the peak concentration of sulphide in the plasma of bile duct ligated rats given a parenteral dose of sulphinpyrazone was 66% of that in normal animals.

The data presented in this paper are in contrast to those reported for the conversion of sulindac (*cis*-5-fluoro-2-methyl-1-[*p*-(methylenesulphinyl)-benzylidene]-indene-3-acetic acid) to sulphide and sulphone metabolites in the rat [16]. Sulindac was reduced rapidly to the sulphide (peak plasma level at 2 hr), whereas the peak plasma level of the sulphone was not detected until about 24 hr after the dose. The sulphide was metabolised extensively to both sulphoxide and sulphone, but the sulphone was not reduced back to the sulphoxide. Since it has been shown that guinea pig liver microsomes have sulphoxide reductase activity (possibly related to NADPH–cytochrome *c* reductase) that can reduce sulindac [6], it appears that the SO moiety of sulindac is reduced in the liver whereas that of sulphinpyrazone is reduced by the gut flora.

These results may have a number of important implications for the clinical use of sulphinpyrazone for its anti-platelet aggregatory properties. Published studies in human subjects [17] have shown that the time-course of the formation of the sulphide from sulphinpyrazone is similar to that occurring in the rat. We are currently studying the role of the gut in the formation of this active metabolite in patients.

Acknowledgements—We are grateful to Ciba–Geigy and the Wessex Medical School Trust for the financial support of these studies.

REFERENCES

1. J. S. Wiley, C. N. Chesterman, F. J. Morgan and P. A. Castaldi, *Thromb. Res.* **14**, 23 (1979).
2. The Anturan Reinfarction Trial Research Group, *New Engl. J. Med.* **298**, 289 (1978).
3. L. Brunner, J. Stepanek and H. Brunner, *J. Pharm. Pharmac.* **32**, 714 (1980).
4. W. Dieterle, J. W. Faigle and J. Moppert, *Arzneimittel-Forsch./Drug Res.* **30**, 989 (1980).
5. A. Kirstein Pedersen and P. Jakobsen, *Br. J. clin. Pharmac.* **11**, 597 (1981).
6. S. Kitamura, K. Tatsumi and H. Yoshimura, *J. Pharm. Dyn.* **3**, 290 (1980).
7. D. M. Gardner and A. G. Renwick, *Xenobiotica* **8**, 679 (1978).
8. J. H. Weisburger, P. H. Grantham, R. E. Horton and

E. K. Weisburger, *Biochem. Pharmac.* **19**, 151 (1970).
9. P. Jenner, J. W. Gorrod and A. H. Beckett, *Xenobiotica* **3**, 341 (1973).
10. R. Gingell, J. W. Bridges and R. T. Williams, *Xenobiotica* **1**, 143 (1971).
11. J. P. Moody and R. T. Williams, *Fd Cosmet. Toxic.* **2**, 707 (1964).
12. A. G. Renwick, in *Presystemic Drug Elimination* (Eds. C. F. George, D. G. Shand and A. G. Renwick) pp. 3–28. Butterworth, London (1982).
13. F. Varga, *Digestion* **14**, 319 (1976).
14. W. Dieterle and J. W. Faigle, *Xenobiotica* **11**, 559 (1981).
15. P. C. Hirom, P. Millburn, R. L. Smith and R. T. Williams, *Biochem J.* **129**, 1071 (1972).
16. D. E. Duggan, K. F. Hooke, R. M. Noll, H. B. Hucker and C. G. van Arman, *Biochem. Pharmac.* **27**, 2311 (1978).
17. P. Jakobsen and A. Kirstein Pedersen, *J. Chromat* **223**, 460 (1981).