that the sex difference seen for lignocaine *N*-deethylase in microsomes [20] is only fully expressed if the correct lipid mixture (i.e. lipids derived from male microsomes) is used with the male-derived enzymes. The data presented in this paper do not agree, therefore, with the view that all sex differences in drug metabolism are a consequence of differences in cytochrome P-450 isozyme content as expressed by Kamataki *et al.* [24] and others (for review, see ref. 25).

In all experiments it was seen that microsomal lipids are more efficient than DLPC in reconstituting *N*-deethylase activity and male-derived lipids gave a higher activity than DLPC:DLPE mixtures. This agrees with earlier findings using partially purified enzymes [7] and cytochrome P-450LM$_2$ [26]. The microsomal lipids as prepared contain a complex mixture of phospholipids, as well as triglycerides and cholesterol and any of these components could account for the effects seen. Indeed, cholesterol, added to DLPC vesicles, has been shown to influence the metabolism of drugs [27]. Sex differences also exist in the composition and fatty acid content of phospholipids in hepatic endoplasmic reticulum [28] and these could lead to the effects seen.

Further work is in progress to separate and analyse the microsomal lipids used in these experiments and to use the separated fractions in reconstitution experiments to ascertain the exact nature of the protein–lipid interaction seen in this study.

In summary, the *N*-deethylation of lignocaine catalysed by a purified isozyme of cytochrome P-450 from male rat liver is greater when reconstituted into microsomal lipids than either DLPC or DLPC:DLPE mixtures. Microsomal lipids derived from the male are more efficient than female-derived lipids in reconstituting this male-specific activity. Sex differences in lignocaine metabolism [20] may, thus, be partially due to protein–lipid interactions in the endoplasmic reticulum and not solely to the presence of sex-specific cytochrome P-450 isozymes.

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Role of the intestinal flora in the acetylation of sulfasalazine metabolites

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A "phase II" reaction of xenobiotic metabolism [1] is known to occur in the liver as well as at such mammalian sites as the intestinal mucosa [2, 3]. Sulfapyridine (SP)* and 5-aminosalicylate (5-ASA), the two primary metabolites of sulfasalazine, are typical substrates for the acetylation reaction; the acetylated derivatives of these metabolites (Ac-SP and Ac-5-ASA) are found in the excreta of animals dosed with sulfasalazine [4, 5]. In this communication, we describe experiments which indicate that the intestinal bacteria of rats, guinea pigs, dogs and humans are capable of acetylating both 5-ASA and SP.

Male Hartley guinea pigs and conventional and germfree male CD rats (Charles River Breeding Laboratories, Wilmington, MA) were maintained in metabolism cages on, respectively, either guinea pig chow (Ralston Purina Co., St. Louis, MO) or germfree rat diet (Charles River Breed-

* Abbreviations: 5-ASA, 5-aminosalicylate; Ac-5-ASA, 5-acetamidosalicylate; Ac-SP, *N*-acetylsulfapyridine; and SP, sulfapyridine.

ing Laboratories) as previously described [6]. Mongrel dogs were maintained on a diet of Respond 2000 (Pro Pet Inc., Syracuse, NY). The human volunteers were healthy men between the ages of 30 and 40 years who gave informed consent according to the guidelines of the Harvard Medical School Committee on Human Studies.

To assay the *in vitro* bacterial acetylation of 5-ASA and SP, recently passed feces or cecal contents obtained from sacrificed animals were suspended (1:20, w/v) in anaerobic [7] 0.1 M KPO_4 buffer at pH 7.0 and incubated at 37° with 5-ASA (1 mM), sulfasalazine (1 mM), SP (1 mM), or in the absence of such substrates. After incubation under anaerobic conditions for 24 hr, the reaction mixtures were clarified by centrifugation (800 *g*), filtered (0.45 μm Millex, Millipore Corp., Bedford, MA), and analyzed as described below.

To assess the *in vivo* acetylation of 5-ASA, six male Hartley guinea pigs (200–250 g) and five male CD rats (175–200 g) were allowed access *ad lib.* to their respective diets which also contained 1% sulfasalazine. The drug-supplemented diet was consumed for 4 days, and the consumption of sulfasalazine calculated from the daily food intake. Urine and feces were collected daily beginning 2 days before the 4-day period of drug administration and ending 3 days after the termination of drug administration. The daily collection of feces was homogenized in a Waring blender, and a 2-g aliquot was extracted three times with 8-ml portions of 0.1 M KPO_4 buffer, pH 6.0. The extracts were pooled, diluted to 25 ml, and filtered. These extracts, as well as filtered and appropriately diluted urine samples, were then analyzed as described below. Recoveries of 5-ASA and Ac-5-ASA added to feces were 75% and greater than 95% respectively.

5-ASA was quantified as its *N*-propionyl derivative, which was formed in a 1-ml sample of fecal or cecal suspension by stirring the sample vigorously and adding several aliquots of propionic anhydride (25 μl; Fisher Chemical Co., Pittsburgh, PA), followed after 5 min with 10 N NaOH (150 μl) and 5 min later with 10 N HCl (150 μl).

HPLC was carried out by means of either a Hewlett–Packard (Palo Alto, CA) model 1080A or a Waters Associates (Milford, MA) model ALC/GPC 204 liquid chromatograph using a 5 μm C-18 reverse phase column (25 cm by 4.6 mm, Supelco, Bellefonte, PA), at 40°. The solvent system was isocratic for 3 min, the eluent consisting of 5% methanol in 0.1 M KPO_4 buffer, pH 2.0, at a flow rate of 1.5 ml/min. A linear gradient was then applied that in 20 min had brought the methanol to a concentration of 50%, at which it remained for 5 min. Using this system, Ac-5-ASA was eluted at 8.7 min and *N*-propionyl-5-ASA at 10.0 min, while SP eluted at 10.7 min, Ac-SP at 13.5 min, and sulfasalazine at 22.6 min. The column was then regenerated, first with a linear gradient that reached 5% methanol within 5 min, and then with an additional isocratic elution with 5% methanol for 5 min.

N-Propionyl-5-ASA, Ac-5-ASA, SP and Ac-SP were detected and quantified in comparison with standards by their absorbance at 225 nm using a Hewlett–Packard 1040A Diode Array Detector, an instrument that also provided a spectrum which enabled the identity of the compound to be confirmed.

Ac-SP, *N*-propionyl-5-ASA and Ac-5-ASA were prepared as previously described [3]. Sulfasalazine was a gift from Pharmacia (Piscataway, NJ), and 5-ASA a gift from Rowell Laboratories (Baudette, MN). SP was purchased from the Sigma Chemical Co. (St. Louis, MO).

When sulfasalazine was incubated with suspensions of guinea pig cecal contents (Table 1), four metabolites appeared whose retention times and respective spectra were the same as those of 5-ASA, Ac-5-ASA, SP, and Ac-SP. 5-ASA seemed to be acetylated more readily than SP when sulfasalazine was the substrate, in spite of the fact that the recovery of SP was greater than that of 5-ASA. In similar experiments, it was found that feces from rats, dogs and humans also acetylated 5-ASA in yields that ranged between 1.9 and 3.4%. It is not clear whether the two acetylation reactions are carried out by the same intestinal bacteria.

To verify that the intestinal bacteria are responsible for the acetylation of 5-ASA, the reaction was compared in fecal suspensions obtained from germfree and conventional rats. Conventional rat feces were found to acetylate 5-ASA to the extent of 1.1% (0.62 to 1.40), whereas no acetylating activity was found in feces from germfree rats. Furthermore, low speed configuration of the fecel suspension (800 *g*) resulted in a pellet that retained most of the capacity to acetylate 5-ASA, lending further support to the conclusion that the reaction is mediated by the intestinal bacteria.

Although the abilities of fecal bacteria from various species to acetylate 5-ASA seem similar, there are species differences in the recovery of Ac-5-ASA in the feces (Table 2). Thus, when sulfasalazine (1%) was added to the diet, the total recovery of both 5-ASA and Ac-5-ASA in rat feces was 53.7%, whereas it was only 18.4% in guinea pig feces. Furthermore, less than half of the 5-ASA recovered in the feces of the rat had been acetylated, whereas in the guinea pig the great majority (85%) was acetylated. Therefore, in terms of the 5-ASA content of the administered sulfasalazine, the recovery of free 5-ASA in the feces of the rat (29.8%) was approximately ten times greater than that in the guinea pig (2.6%).

Such findings suggest that acetylation by the flora may determine the extent to which 5-ASA, the active moiety of sulfasalazine [8], is acetylated at its site of action in the colon. The point may have clinical significance as one [9] of two studies [9, 10] indicates that enemas of Ac-5-ASA are not effective in treating ulcerative colitis, whereas enemas of 5-ASA are effective [11–14].

It remains to be determined, therefore, whether the capacity of the bacteria of the flora to acetylate 5-ASA, which has been demonstrated in these studies, may influence the clinical effectiveness of 5-ASA.

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Table 1. *N*-Acetylation of sulfasalazine and its metabolites by cecal and fecal preparations from the guinea pig*

Substrate	5-ASA	Ac-5-ASA	SP	Ac-SP
	Cecal contents (% Yield)			
Sulfasalazine†	32.1	2.21	84.7	0.84
	40.8	2.73	98.4	0.87
5-ASA	31.1	2.72		
	34.5	3.10		
SP			75.5	0.85
			77.5	0.89
	Feces (% Yield)			
Sulfasalazine¶	15.4	1.05	62.2	0.06
	16.0	1.09	63.4	0.08
5-ASA	36.2	0.95		
	29.9	1.11		
SP			85.0	0.38
			86.0	0.50

* Incubation conditions were as described in the text. Results from each of two experiments are shown.

† Recovery of sulfasalazine was 0.02% in these experiments.

¶ Recovery of sulfasalazine was 16% in these experiments.

Table 2. Recovery of 5-ASA and Ac-5-ASA following the administration of dietary sulfasalazine to rats and guinea pigs*

	Recovery (%)			
	Urine†	Feces		Total
	Ac-5-ASA	5-ASA	Ac-5-ASA	
Rats	23.1 ± 2.0	29.8 ± 2.4	23.9 ± 3.3	76.7 ± 5.3
Guinea pigs	36.0 ± 3.6	2.6 ± 1.0	15.8 ± 2.0	54.3 ± 4.7

* Five rats received a diet containing 1% sulfasalazine from day 1 until day 4, which gave the individual animals doses equivalent to 213, 232, 238, 269 and 279 mg of 5-ASA during the 4-day period. Six guinea pigs received a diet containing 1% sulfasalazine from day 1 until day 4, which gave the individual animals doses equivalent to 120, 129, 132, 134, 159 and 162 mg of 5-ASA during the 4-day period. Total recovery is expressed as the mean ± SD in terms of the percentage of 5-ASA contained in the administered sulfasalazine.

† 5-ASA was not detected in the urine of any animal.

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Oxygen radical injury in the presence of desferal, a specific iron-chelating agent

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Oxygen radicals and activated oxygen species, products of biological reduction of oxygen, have been implicated in the pathophysiology of tissue injury due to ischemia-reperfusion, side effects of drugs, irradiation and inflammation [1, 2]. One of the important chemical reactions for the formation of these toxic species was proposed in 1934 by Haber and Weiss [3], as shown below:

$$H_2O_2 + O_2^{\cdot-} \rightarrow OH^- + OH^{\cdot} + O_2 \quad (I)$$

This reaction is thermodynamically feasible and has been documented as a source of the highly toxic hydroxyl radical ($OH^{\cdot}$) [4–6]. However, under chemically well-defined conditions, the reaction has been suggested to be kinetically very slow or even negligible [7, 8]. the overall stoichiometry of the often cited Haber–Weiss reaction is widely accepted, but the reaction has been suggested to be a combination of the following two half-reactions requiring iron as the catalyst as described below [8]:

$$O_2^{\cdot-} + Fe^{3+} - EDTA \rightarrow O_2 + Fe^{2+} - EDTA \quad (II)$$

$$Fe^{2+} - EDTA + H_2O_2 \rightarrow Fe^{3+} - EDTA + OH^- + OH^{\cdot} \quad (III)$$

$$H_2O_2 + O_2^{\cdot-} \rightarrow OH^- + OH^{\cdot} + O_2 \quad (I)$$

Requirement of iron in reactions II and III for the production of $OH^{\cdot}$ (reaction I) has also been demonstrated in *in vitro* hydroxylation studies [9]. Hydroxylation of aromatic compounds with xanthine–xanthine oxidase, at pH 7.4, could not occur until low concentrations (μM range) of $FeSO_4$ or $FeCl_3$ were provided in the medium [9]. The