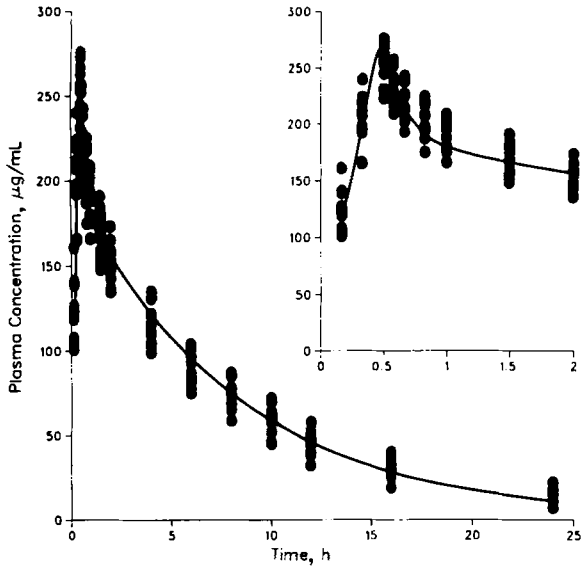

Figure 1—*All ceftriaxone plasma concentration–time data from 12 subjects together with the curve fitted using the composite pharmacokinetic profiling technique and three subjects in each of four sampling groups, as depicted in Table IV (inset).*

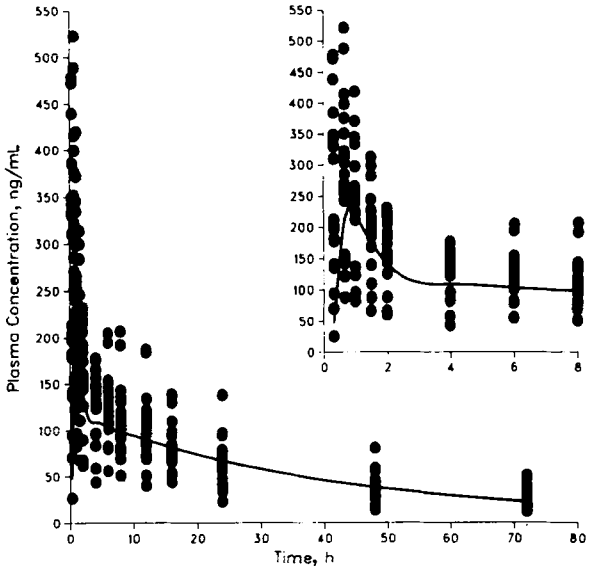

Figure 2—*All diazepam plasma concentration–time data from 20 subjects together with the curve fitted using the composite pharmacokinetic profiling technique and five subjects in each of four sampling groups, as depicted in Table IV (inset).*

subjects. Blood samples were obtained at specific times over the following 72 h. The subjects were divided into four groups of five subjects each: group I was sampled at 0, 0.33, 2, 12, and 72 h; group II was sampled at 0, 0.67, 4, 16, and 72 h; group III was sampled at 0, 1, 6, 24, and 72 h; group IV was sampled at 0, 1.5, 8, 48, and 72 h after the oral dose. The analytical *CV* for this study was 11.3%.

Four curve-fitting techniques were used to evaluate the data: (*a*) concentration–time data from the individual subjects were fitted and the mean parameters were calculated; (*b*) a single mean concentration–time profile from all subjects was fitted to determine parameter values; (*c*) all concentration–time data were fitted simultaneously to determine the parameter values; (*d*) a limited sample composite concentration–time profile was fitted to determine the parameter values. The results of these fitting procedures are presented in Table IV. In the first three of the methods, all of the concentration–time data were used in the sampling/fitting procedure, whereas in the final method, four groups of three or five patients, respectively, were used for ceftriaxone and diazepam. There was good general agreement of parameter estimates obtained by each of the four sampling/fitting techniques. The parameters A and α were variable among the four sampling/fitting procedures, and for the oral absorption of diazepam, k_a was variable as well. However, the remaining parameters for both diazepam and ceftriaxone were extremely consistent among the four methods. Again it should be noted that the clearances, volumes of distribution, and elimination rate constants show little variability. All ceftriaxone and diazepam concentration–time points, together with the composite profiling fits, are presented in Figs. 1 and 2, respectively, to show the randomness of scatter around the fitted curves.

The results of these simulations and curve-fitting procedures indicate that the composite pharmacokinetic profiling technique developed herein is a useful method that can be utilized to minimize the number of blood samples and, therefore, the total blood volume withdrawn. The application of this technique can allow for the development of pharmacokinetic profiles in patient populations, including neonates and certain disease states, in which little information has been generated due to sampling limitations. This technique has been used to develop a preliminary pharmacokinetic profile of vitamin E following an intramuscular injection to premature neonates (3).

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Disposition of Nitrofurantoin and Nitrofurazone in the Isolated Perfused Rat Kidney

Keyphrases □ Nitrofurantoin—renal metabolism, isolated perfused rat kidney □ Nitrofurazone—renal metabolism, isolated perfused rat kidney

To the Editor:

Nitrofurantoin is frequently used clinically to treat urinary tract infections, but little is known of its renal metabolism. The side effects of nitrofurantoin therapy, pulmonary and hepatic toxicities, and polyneuropathies, are thought to be a consequence of its reductive metabolic activation (1–5). The end

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products of this activating pathway are the 5-amino and cyano derivatives (6–8). Both of these metabolites have been found following anaerobic metabolism of nitrofurantoin by the 9000×*g* supernatant of rat liver and kidney homogenates (9). In addition, the cyano metabolite was detected in the perfusate and bile of isolated perfused rat livers (10), and the 5-amino derivative has been detected in the urine of male volunteers receiving an intravenous infusion or an oral tablet of nitrofurantoin (11). A third metabolite, the 4-hydroxy derivative, resulting from hydroxylation of the furan ring, has been identified (12) and detected in the urine of rats and the media of isolated perfused livers of rats induced with 3-methylcholanthrene (13–15). Unchanged nitrofurantoin in the urine accounted for 34–47% of the dose in humans (11) and 16–52% of the dose in rats (16–19).

Nitrofurazone, which is structurally similar to nitrofurantoin, is used topically as an antibacterial agent, but the frequency and severity of side effects preclude its systemic use. It is also reduced under anaerobic conditions to the analogous 5-amino and cyano derivatives (20). Furthermore, after subcutaneous injection, nitrofurazone produces kidney and liver lesions in the rat (21). Only 5–8% of a nitrofurazone dose was excreted unchanged in the urine of rats (16, 17, 22).

The available evidence suggests that, at least under *in vitro* anaerobic conditions, both nitrofurantoin and nitrofurazone are metabolized by kidney preparations. However, whole animal studies suggest that nitrofurazone is both more extensively metabolized and more toxic than nitrofurantoin.

We have studied the disposition of these two 5-nitrofurans in the isolated perfused kidney. We were particularly interested in studying the disposition of nitrofurantoin by the kidney because it controls the access of the drug to the urinary tract, and hence, the site of infection. The intact organ system allowed us to study the metabolism of nitrofurantoin by the kidney under well-oxygenated conditions without the potential confounding aspects of extrarenal, particularly hepatic, metabolism. Particular attention was directed to the known reduced metabolites of nitrofurantoin, since their presence would indicate that metabolic activation had occurred. Nitrofurazone was selected for comparison because it is structurally similar and metabolically activated to analogous derivatives; it is more toxic in general, and to the kidney in particular, than nitrofurantoin.

For each antibiotic, six rat kidneys were perfused with 6.5% bovine serum albumin dissolved in a Krebs–Henseleit buffer supplemented with glucose and eight amino acids (23). Inulin was added as a marker of the glomerular filtration rate. The 70 mL of recirculating perfusate was aerated with 95% O_2–5% CO_2, and the pH and temperature were maintained at 7.4 and 37°C, respectively. After a 20-min equilibration period, 0.1 mM nitrofurantoin or nitrofurazone dissolved in perfusate was added to the reservoir. Perfusate and urine were sampled with replacement at frequent intervals for 180 min. The pO_2 and pH were determined using a blood gas analyzer[1] and Na^+/K^+ levels were measured with a flame photometer[2]. Inulin was quantitated spectrophotometrically (24). Nitrofurantoin and nitrofurazone and their amino and cyano metabolites were quantitated by HPLC (25, 26). For nitrofurantoin the reabsorption of Na^+, fractional excretion of K^+, and the glomerular filtration rate were 90.0 ± 3.1%, 0.86 ± 0.28, and 0.39 ± 0.15 mL/min, respectively[3]. For nitrofurazone the corresponding values were 95.8 ± 1.4%, 0.49 ± 0.16, and 0.22 ± 0.07 mL/min. Although these glomerular filtration rates are low (27), suggesting a compromised preparation, subsequent studies in kidneys with improved physiological function handled both 5-nitrofurans similarly to the results reported here[4].

The concentration in the perfusate of both nitrofurantoin and nitrofurazone could be described by a one-compartment body model with first-order elimination kinetics. The total clearances[5], 0.64 ± 0.12 and 0.75 ± 0.18 mL/min for nitrofurantoin and nitrofurazone, respectively, were not significantly different. However, the fractions excreted unchanged in the urine[6] after 3 h, 0.19 ± 0.10 for nitrofurantoin and 0.02 ± 0.02 for nitrofurazone, were significantly different ($p < 0.005$). Known reduced metabolites of nitrofurantoin, the 5-amino and cyano derivatives, while not detectable in the perfusate, accounted for ~3% of the dose in the urine. Neither the 5-amino nor the cyano metabolite of nitrofurazone was detected in either the perfusate or urine. There was no evidence that the 4-hydroxy derivative of either 5-nitrofuran was formed. There appears to be net tubular reabsorption of both 5-nitrofurans since the ratios of their renal clearance to the glomerular filtration rate are <1.0 (0.39 ± 0.20 and 0.07 ± 0.06 for nitrofurantoin and nitrofurazone, respectively). These ratios could be <1.0 in the absence of tubular reabsorption if the 5-nitrofurans were highly bound to bovine serum albumin. However, only 60% of nitrofurantoin is bound to plasma proteins (11). Furthermore, nitrofurantoin, which has a pK_a of 7.2, is known to be secreted by the organic anion transport system and to be reabsorbed (28). Certainly at the pH of the urine (6.0–6.5), nitrofurantoin would be mostly un-ionized and could be reabsorbed by passive diffusion. Nitrofurazone is not ionized at the pH values encountered in this study.

At the conclusion of the 3-h experiment most of the dose of both these 5-nitrofurans is unaccounted for. Less than 15% remains in the perfusate as unchanged drug; 19% of the nitrofurantoin was excreted unchanged in the urine and ~3% as known metabolites. Thus, ~60% of the dose of nitrofurantoin is unaccounted for. For nitrofurazone, only 2% of the dose was detected in the urine. Hence, ~85% of the dose of nitrofurazone is unaccounted for. For both of these 5-nitrofurans, the fraction unaccounted for may be drug bound to tubing or chemically degraded, drug or metabolites trapped intracellularly, as yet unidentified metabolites, and/or drug covalently bound to tissue biomacromolecules after metabolic activation. The first possibility was dismissed, since identical 3-h experiments without the kidney in place resulted in no loss of either 5-nitrofuran. Some preliminary work with the 5-[2-^{14}C]nitrofurans has shown that nitrofurantoin, nitrofurazone, or their known metabolites were not detected after exhaustive extraction of homogenates of the perfused kidneys first with acetonitrile–water and then 5% trichloroacetic acid[4]. Thus, although the ^{14}C-label was found in the tissue, it would appear that neither the parent compound nor known metabolites were trapped intracellularly. Each of the remaining alternative hypotheses involve metabolism of the 5-nitrofurans. Since both the 5-amino and cyano derivatives of nitrofurantoin were detected in the urine, the drug must have been metabolically activated, and at least some of it could, therefore, have been

[1] Model C-175; Corning, Corning, N.Y.
[2] Model 450; Corning.

[3] All data is reported as mean ±*SD*, $n = 6$.
[4] B. Hoener, T. V. Zenser, B. B. Davis, and U. Bredberg, unpublished results.
[5] Dose/area under the curve.
[6] Cumulative ampunt excreted in urine/dose.

bound to biomacromolecules rather than have been further metabolized and eliminated as the end products of the activating pathway. Our preliminary work also indicates that ~5% of the ^{14}C-label is covalently bound to biomacromolecules at the end of the 3-h experiment[4]. Others have found carbon-14 bound to biomacromolecules after incubation of [2-^{14}C]nitrofurantoin with microsomal preparations from rat liver and lung (29). It is probable, therefore, that at least some portion of the dose of nitrofurantoin is covalently bound to the biomacromolecules in the perfused rat kidney with the remainder eliminated by as yet unidentified metabolic pathways. Since none of the nitrofurazone was excreted as the 5-amino or cyano metabolite, there is no direct evidence that any of the drug was reductively activated for binding to biomacromolecules. Other investigators have, however, found ^{14}C-label covalently bound to the proteins, DNA, and RNA, of the liver and kidney of rats fed [2-^{14}C]nitrofurazone (30), and our preliminary studies have found ~5% of the ^{14}C-label bound to biomacromolecules of the nitrofurazone perfused kidney[4]. The remaining 80% of the dose of nitrofurazone was accounted for by as yet unidentified metabolites in the perfusate and urine. These studies indicate that the perfused kidney does metabolize both nitrofurantoin and nitrofurazone. Furthermore, the more toxic nitrofurazone appears to be more extensively metabolized.

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Pharmacokinetic Implications of Stereoselective Changes in Plasma-Protein Binding: Warfarin/Sulfinpyrazone

Keyphrases □ Warfarin—plasma-protein binding, stereoselective displacement, drug-drug interaction with sulfinpyrazone □ Sulfinpyrazone—plasma-protein binding, stereoselective displacement, drug-drug interaction with warfarin

To the Editor:

The intensity of the observed pharmacological response produced by any drug is invariably related, directly or indirectly, to its plasma concentration. In the systemic circulation most drugs are bound to plasma proteins, and thus an equilibrium is established between bound and free drug. Since free drug alone is the pharmacologically active agent, the drug-plasma protein interaction may not only influence overall drug disposition but also affect the magnitude and time-course of the pharmacological response. In theory, numerous models could describe the relationship between hepatic clearance and plasma protein binding. However, a widely accepted model which has been shown to explain the vast majority of empirical observations, and one which is consistent with the results to follow, is the so-called well-stirred model (1-4). For drugs exhibiting a low extraction ratio (ER <0.3), it may be shown that their observed total body clearance (CL) is directly related to their unbound fraction within plasma (f_u) (5, 6). Consequently, for low-extractration ratio drugs which are highly protein bound (>98%), a small change in the fraction of the drug bound to plasma protein will not only result in a relatively large change in f_u but also in a proportional change in CL and may thus have potentially important consequences in the in-

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