shown by subjecting the tablets to *in vitro* test conditions in which the intensity of agitation was very much less than exists during performance of the USP tablet disintegration test. The flask and stirrer method of Poole (1969), as described by Wagner (2), was employed. For the first 0.5 hr., the tablets were subjected to 0.1 N HCl with a stirring rate of 50 r.p.m. For the remainder of the test, the tablets were stirred in pH 6.9 buffer, first at a stirring rate of 50 r.p.m. and then at a stirring rate of 200 r.p.m. Three of the enteric-coated tablets tested under these conditions exhibited initial cracks in the coatings after 230, 290, and 300 min. at 50 r.p.m. and then slowly released 22.7, 24.0, and 24.0% of the labeled dose over additional periods of 160, 60, and 40 min., respectively. When the stirring rate was increased to 200 r.p.m., these tablets eventually released essentially the labeled dose of drug. A fourth tablet, tested under the same conditions, showed no release of drug after 420 min. at 50 r.p.m.

Under the same conditions, the sodium aminosalicylate solution, when added to acid such that the final concentration was 0.1 N HCl, showed no precipitation of aminosalicylic acid (*i.e.*, infinite rate of dissolution). The suspension gave apparent first-order dissolution in 0.1 N HCl at 50 r.p.m., with a rate coefficient of 4.28 hr.$^{-1}$; at the end of 30 min., an average of 89.0% of drug was in solution (average of five tests). The compressed tablet similarly released drug in 0.1 N HCl at 50 r.p.m., with a coefficient of 2.58 hr.$^{-1}$; at the end of 30 min., an average of 71.3% of the labeled dose of drug was in solution (average of four tests with individual tablets). The enteric-coated tablets did not disintegrate in the classical sense when stirred at 50 r.p.m. in the pH 6.9 buffer. The coatings merely cracked open, and the drug was released extremely slowly from the ruptured tablets. It was not until the stirring rate was increased to 200 r.p.m. that the tablets really disintegrated and eventually released their labeled content of drug. These results strongly suggest that the intensity of agitation in the USP tablet disintegration test is too intense, at least for enteric-coated aminosalicylic acid tablets, and that the USP test does not predict performance in man. Thus, this is a documented case where a commercial product met compendia standards but was not suitable from a standpoint of effectiveness.

Feldmann (3) stated: "In other words, from a statistical viewpoint, the number of cases in which products have been found to meet compendia standards, but are not suitable from the standpoint of effectiveness or safety, are negligible."

Historically there are several examples which could be used to challenge the above quotation, but in this communication we document an example discovered in our laboratory.

(1) R. G. Stoll, P. D. Holmes, S. B. Roth, and J. G. Wagner, *Res. Commun. Chem. Pathol. Pharmacol.*, 4, 327(1972).

(2) J. G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," 1st ed., Drug Intelligence Publications, Hamilton, Ill., 1971, p. 112.

(3) E. G. Feldmann, Statement of the National Formulary of the American Pharmaceutical Association to the Select Committee on Small Business—Subcommittee on Monopoly of the Senate of the United States, 90th Congress, 1st Session, Washington, D. C., June 8, 1967.

JOHN G. WAGNER▲
PAUL K. WILKINSON
ALLEN J. SEDMAN
ROGER G. STOLL
College of Pharmacy and
Upjohn Center for Clinical Pharmacology
University of Michigan
Ann Arbor, MI 48104

Editor's note: The number of different drug products on the U. S. market has been estimated at somewhere between 100,000 and 300,000. A most conservative estimate would be that one batch or lot has been produced annually of each product over the 5-year period between the quoted testimony and Dr. Wagner's report of the faulty lot of aminosalicylic acid tablets. This amounts to a defect rate of one lot out of a total of $\frac{1}{2}$ million to $1\frac{1}{2}$ million batches of drug products. As Dr. Wagner notes, there have been several such reports, probably raising the number of defects to five or perhaps 10; even this upper figure would give a defect ratio of approximately 1:100,000. We are grateful to Dr. Wagner for confirming our hypothesis that from a statistical viewpoint such incidences indeed are negligible. —EGF

Reliable and Simple Method of Intravenous Injection into the Laboratory Rat

Keyphrases □ Injection techniques, intravenous—use of dorsal penis vein, rats □ Drug administration—intravenous injection *via* dorsal penis vein, rats □ Intravenous injection of drugs into small laboratory animals—dorsal penis vein method

Sir:

In a previous article, Salem *et al.* (1) demonstrated a technique for intravenous injection of drugs into small laboratory animals *via* the dorsal penis vein. The advantages of this procedure compared to the tail vein method (2) lie in its simplicity, rapidity, reproducibility, and ease of injection. Tail vein injections into rats weighing over 100 g. are usually difficult and poorly reproducible, and they generally require a great degree of skill. On the other hand, the dorsal penis vein technique requires very little prior experience, so a relatively untrained laboratory worker can master the procedure in a short time, *i.e.*, one to two trials. In addition, tissue damage at the site of injection will have a minor overall effect on the animal compared to intracardial injection, and surgical manipulations such as those involved with femoral vein injections (2) are not required.

Due to these advantages it is not surprising that this method of drug administration is being employed in biopharmaceutical and pharmacokinetic studies. A review of the literature indicates that little information has been published concerning the physiological fate of drugs administered *via* this route. It is important to know whether this vein is part of the portal or general circulation. Drugs administered orally or intraperitoneally are absorbed *via* the mesentery blood system. These vessels combine to form the portal vein which leads directly to the liver. The drug plasma concentra-

Table I—Urinary Excretion Data of 4-Aminoantipyrine

Mean[a] Param- eters	Route of Injection[b]	
	Intracardial ($\pm$ SE)	Penal Vein ($\pm$ SE)
K_E (hr.)$^{-1}$	0.232 $\pm$ 0.031	0.246 $\pm$ 0.031
$t_{1/2}$(hr.)	3.24 $\pm$ 0.41	3.03 $\pm$ 0.32
R_1[c]	11.14 $\pm$ 0.64	10.94 $\pm$ 1.66
R_2[d]	88.87 $\pm$ 0.64	89.10 $\pm$ 1.65
k_e(hr.)$^{-1}$	0.026 $\pm$ 0.004	0.032 $\pm$ 0.009
k_m(hr.)$^{-1}$	0.201 $\pm$ 0.027	0.246 $\pm$ 0.030
k_e/k_m	0.126	0.130

[a] Mean of six animals. [b] No statistical difference in parameters due to route of injection as determined by the Student t test. [c] R_1 = ratio of cumulative amount excreted at time infinity of unmetabolized to total drug times 100 = k_e/K_E. [d] R_2 = ratio of cumulative amount excreted at time infinity of metabolized to total drug times 100 = k_m/K_E.

tion is relatively high during this first pass through the liver; for substances that are biotransformed at this site, the rate of metabolism can be expected to be higher than when the drug is administered *via* a route leading directly to the heart. This first-pass effect can be associated with an increase in the fraction of drug metabolized. The present study was undertaken to examine the fate of a drug after penis vein injection and to determine the influence of this route of administration on metabolism. In addition, the feasibility of performing urinary excretion studies after penis vein injection was explored.

Male Sprague–Dawley rats[1] were used. The animals (six in each group), weighing 257–268 g., were lightly anesthetized with ether and subsequently injected with a pH 7.4 Sorensons phosphate buffer solution of 4-aminoantipyrine (70 mg./kg.) by either the intracardial or dorsal penis route. Blood and urine samples were collected as a function of time and assayed for both the free drug and the acetylated metabolite according to the method of Brodie and Axelrod (3). Urinary drug recovery after 72 hr. expressed as percent of total dose recovered was 71.9 $\pm$ 4.6 and 73.2 $\pm$ 2.7% for the intracardial and dorsal penis vein injections, respectively.

The cumulative amount of unmetabolized drug excreted in the urine was calculated as a function of time, and the data were appropriately plotted in accordance with the sigma-minus method (4). The overall elimination rate constant (K_E) and the biological half-life ($t_{1/2}$) were determined from the least-squares slopes of such plots. A representative graph is shown in Fig. 1. As expected, the half-life of the drug was identical for both groups, regardless of the administration technique. In addition, the half-life of the drug calculated from blood level data after penis vein injection was essentially similar (2.9 $\pm$ 0.3) to that obtained *via* urinary excretion (3.0 $\pm$ 0.3).

Measurement at time infinity, 72 hr., of the total cumulative amount of unchanged and metabolized drug recovered in the urine allows one to calculate the excretion rate constants for unchanged (k_e) and metabolized (k_m) 4-aminoantipyrine. The data shown in Table I summarize the pharmacokinetic parameters determined from the urinary excretion data. It appears
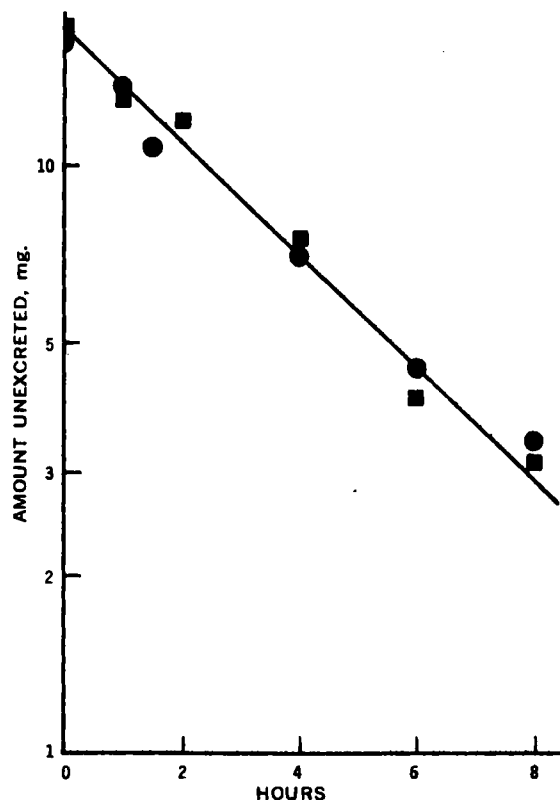

Figure 1—*Mean 4-aminoantipyrine excretion data after a 70-mg./kg. intravenous dose. Key:* ●, *intracardial; and* ■, *dorsal penis vein.*

that the degree of 4-aminoantipyrine metabolism is similar for both intracardial as well as penis vein injection. This suggests that drug injected *via* the dorsal penis vein does not directly enter the portal circulation. This was confirmed by following the suggested procedure of Conway[2], where 0.25–0.50 ml. of air was injected into the penis vein and the air bubbles were observed flowing through the inferior vena cava and not the portal vein. Injection of air into the femoral vein produces the same result.

Based on these experiments, it can be concluded that injection of drug into the dorsal penis vein leads to the general circulation and that a first-pass effect on drug metabolism is not to be expected.

(1) H. Salem, M. H. Grossman, and D. J. Bilbey, *J. Pharm. Sci.*, **52**, 794(1963).

(2) "The Rat in Laboratory Investigation," E. J. Farris and J. Q. Griffith, Eds., Hafner, New York, N. Y., 1967, p. 289.

(3) B. B. Brodie and J. Axelrod, *J. Pharmacol. Exp. Ther.*, **98**, 171(1950).

(4) E. Nelson, *J. Pharm. Sci.*, **50**, 181(1961).

CHARLES H. NIGHTINGALE▲
MICHEL MOURAVIEFF

School of Pharmacy
University of Connecticut
Storrs, CT 06268

[1] Blue Spruce Farms, Altamont, N. Y.

[2] Walter Conway, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, personal communication.