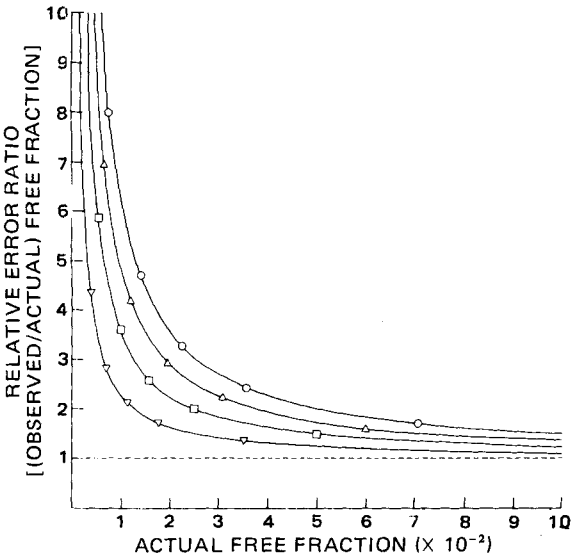

Figure 2—*Effects of varying degrees of radiochemical impurities present in dialysis on the relative error involved in the determination of plasma protein binding of drugs. Key:* ○, *10.0;* △, *7.5;* □, *5.0; and* ▽, *2.5% radiochemical impurities.*

upward shift of the linear relationship between the observed *versus* the actual free fraction.

However, the relative error on the determination of drug protein binding caused by the radiochemical impurities varies markedly depending on the actual free fraction; the lower the actual free fraction the greater the relative error and vice versa. This relative error can be expressed as the ratio of the observed to actual free fraction, which indicates how many times higher the observed free fraction is than the actual free fraction.

Figure 2 shows a plot of this relative error ratio *versus* the actual free fraction. It has an expected hyperbolic form for any given degree of radiochemical impurities present because the independent variable is in the denominator of the dependent variable. It can be seen from Fig. 2 that the observed free fraction will be ~2.3–6.3 times higher than an actual free fraction of 0.01 when radiochemical impurities vary from 2.5–10%, while the observed free fraction is only ~1.1–1.5 times higher than an actual free fraction of 0.1 with the same range of radiochemical impurities present.

The use of a radiolabel containing radiochemical impurities in dialysis for the determination of protein binding in clinical pharmacokinetic studies will not only result in a wrong value, but it will make all comparisons of binding data difficult. Such comparisons are frequently of interest, *e.g.*, when evaluating the effects of other drugs or disease states on the protein binding of drugs or when comparing drug binding in different groups of age or sex. This effect can be illustrated by two groups of drug binding data with average actual free fractions of 0.01 and 0.02. When a radiolabel with 5% radiochemical impurities is used, this 100% difference in the actual free fractions will reduce to a 28% difference in the free fractions, *i.e.*, the observed free fractions will be 0.035 and 0.045. It is likely that a true statistically significant difference among various groups of binding data may therefore become obscured. Because of the significance of drug protein binding in clinical pharmacology, it is suggested that radiolabels used for the determinations of plasma protein binding of drugs be purified prior to dialysis, *e.g.*, by high-pressure liquid chromatography, and that the stability of the radiolabel be verified postdialysis in both buffer and plasma phases.

(1) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).

(2) G. Levy, in "The Effect of Disease States on Pharmacokinetics," L. Z. Benet, Ed., American Pharmaceutical Association, Washington, D.C., 1976, p. 137.

(3) T. D. Bjornsson, P. J. Meffin, S. Swezey, and T. F. Blaschke, *J. Pharmacol. Exp. Ther.*, **210**, 316 (1979).

(4) A. H. Anton, *ibid.*, **129**, 282 (1960).

(5) D. G. McDevitt, M. Frisk-Holmberg, J. W. Hollifield, and D. G. Shand, *Clin. Pharmacol. Ther.*, **20**, 152 (1976).

(6) G. Levy, *J. Pharm. Sci.*, **65**, 1264 (1976).

(7) R. H. Cotham and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 535 (1974).

(8) O. Borgå, K. M. Piafsky, and O. G. Nilsen, *ibid.*, **22**, 539 (1977).

(9) M. Wood, D. G. Shand, and A. J. J. Wood, *ibid.*, **25**, 103 (1979).

(10) K. M. Giacomini, S. E. Swezey, J. C. Giacomini, and T. F. Blaschke, *Life Sci.*, **27**, 771 (1980).

(11) A. Yacobi and G. Levy, *J. Pharmacokinet. Biopharm.* **3**, 439 (1975).

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Determination of Renal Clearances Using Arterial and Venous Plasma: Procainamide in Rabbits

Keyphrases □ Procainamide—renal clearance, determination using timed-interval method, rabbits □ Renal clearance—determination of procainamide using arterial and venous plasma, rabbits □ Pharmacokinetics—determination of renal clearance of procainamide using arterial and venous plasma, rabbits

To the Editor:

The timed-interval method is commonly employed in pharmacokinetic studies to determine the renal clearance (Cl_r) of a drug compound:

$$Cl_r = \frac{X_{t_1-t_2}}{AUC_{t_1-t_2}} \qquad \text{(Eq. 1)}$$

where $X_{t_1-t_2}$ and $AUC_{t_1-t_2}$ are the amount of the intact drug excreted and the area under the plasma concentration curve between times t_1 and t_2, respectively. To date, the potential effect of the source of plasma data, either arterial or venous, on the estimation of clearance using Eq. 1 has rarely been evaluated. The arterial and venous differences of six compounds were recently reported (1). The present

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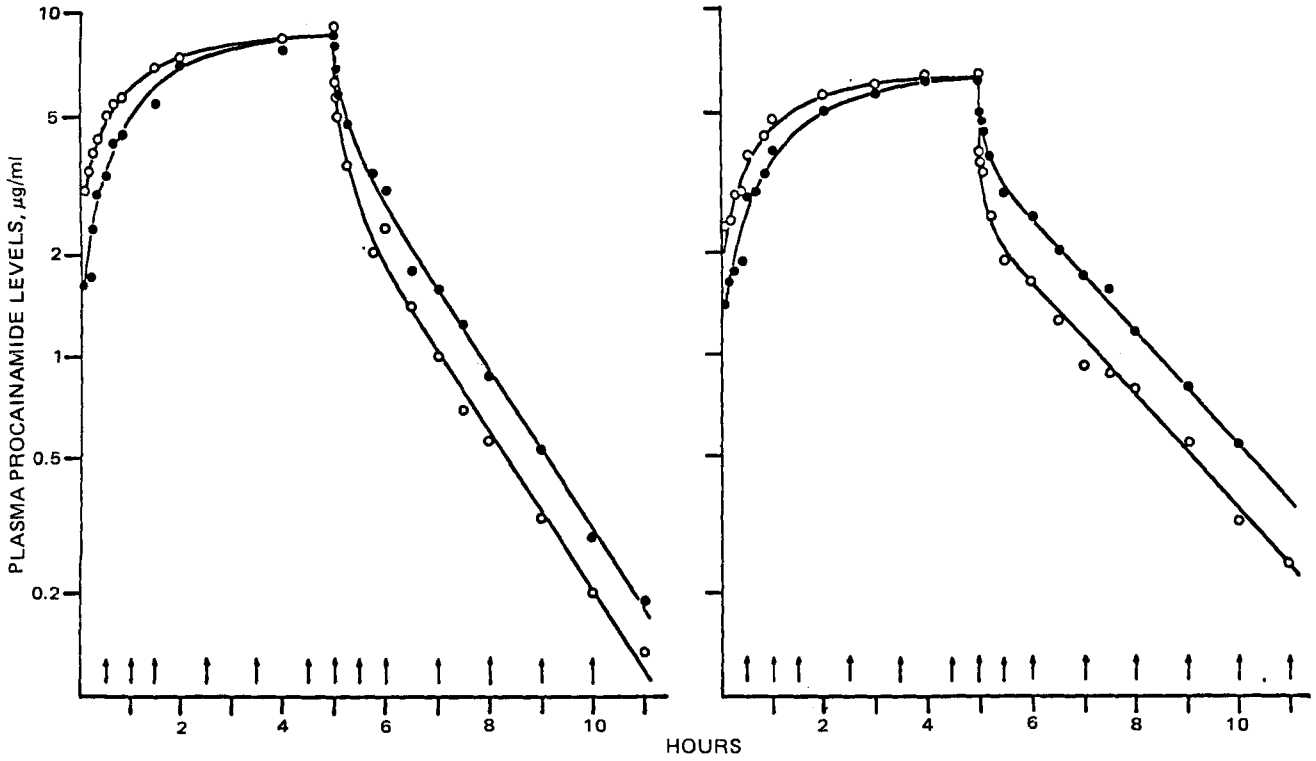

Figure 1—*Arterial (○) and venous (●) plasma procainamide concentration following constant intravenous infusion of procainamide hydrochloride, 52.4 and 92.4 mg/hr for 5 hr into rabbit 1 (left) and rabbit 2 (right), respectively. The arrows indicate the end of each renal clearance determination period.*

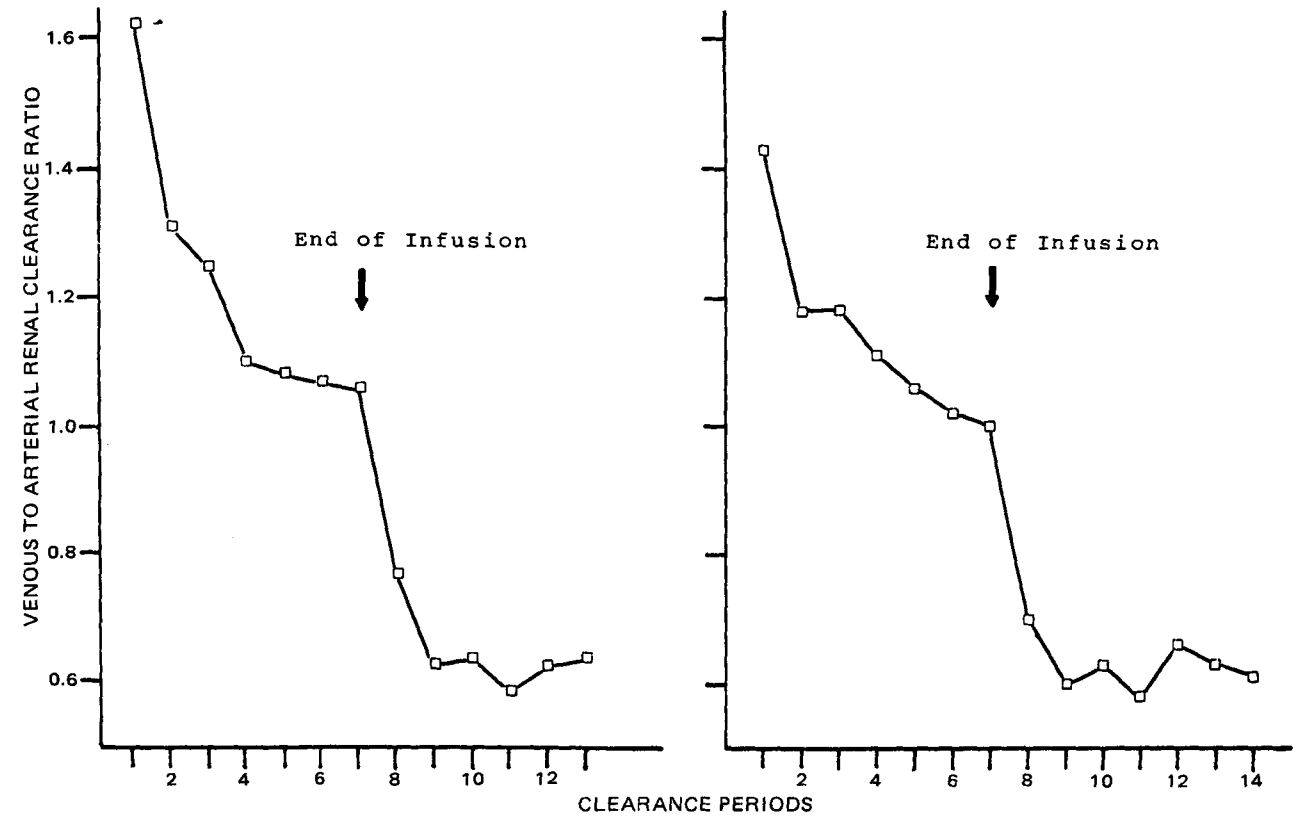

Figure 2—*Venous to arterial renal clearance ratios during and after constant intravenous infusion of procainamide hydrochloride in rabbit 1 (left) and rabbit 2 (right). The abscissa represents the number of renal clearance determination periods.*

communication reports the preliminary results of the influence of arterial–venous (A–V) differences on the determination of renal clearance of procainamide in rabbits using the described method during and after intravenous infusion.

The right carotid artery and the jugular vein of two pigmented male rabbits were catheterized with polyethylene tubings after anesthetization with 1.0 g of urethan/kg[1]. A third catheter was placed into the sacral part of the vena cava *via* the right femoral vein. The tip of the cannula was positioned at the junction of the femoral branch and was confirmed after termination of the experiment. The animals were allowed to recover overnight. Infusion solutions were prepared by dissolving varied amounts of procainamide hydrochloride[2] in normal saline before the study and were administered to the rabbits by a constant-infusion syringe pump[3] into the jugular vein for 5 hr. Arterial and venous blood were collected simultaneously from the carotid artery and femoral vein together with urine samples at predetermined times during and after infusion. A pediatric Forley catheter was introduced into the urinary bladder for collection of urine. Distilled water (3 × 20 ml) was flushed into the bladder shortly before each collection time. An aliquot from the pooled urine and washings was collected. Plasma and urine procainamide concentrations were determined by a modified HPLC assay (2).

The plasma profiles of the two rabbits are shown in Fig. 1. During the infusion arterial plasma levels rose rapidly and appeared to reach steady state at 5 hr. Venous plasma levels, however, were lower than the arterial levels at all times but approached them asymptotically toward the end of the infusion. Both curves began to drop with the venous curve being higher than the arterial curve shortly after the infusion. During the terminal phase, both curves decayed virtually parallel to each other with mean venous to arterial (V/A) ratios of ~1.6 for both rabbits.

Renal clearances were determined by Eq. 1 and the resultant V/A clearance ratios are shown in Fig. 2. Renal clearance measurements using venous data resulted in persistently higher values during infusion and lower values postinfusion than arterial data.

By definition, renal clearance is defined as the virtual volume of arterial plasma flowing into the kidney completely cleared of drug per unit of time. The drug concentrations in the systemic arterial plasma can be considered practically identical at any time. Therefore, it seems only appropriate that Eq. 1 is used with arterial plasma data, unless there is no significant A–V difference. Our results suggest that venous data gave rise to overestimation of renal clearance during infusion and underestimation postinfusion. At steady state, both arterial and venous plasma levels appeared to be identical (Fig. 1); hence, no difference in renal clearance estimate is to be expected from using either data.

The problem of venous sampling in clearance measurements was pointed out earlier (3) and was the subject of a recent review (4). Since relatively little effort has been directed toward the differentiation of arterial and venous blood data in pharmacokinetic analysis, our data provided evidence of the existence of significant A–V differences (Fig. 1) and its consequences (Fig. 2) in renal clearance estimations. It is our opinion that sampling arterial plasma is preferred in renal clearance studies in animals using the described timed-interval method. Nevertheless, venous sampling could be useful when employing steady-state infusion or multiple dosing (area during a dosing interval at steady state) methods. The latter methods should be of special value in human studies where the risk of arterial sampling can be avoided.

(1) W. L. Chiou, G. Lam, M. L. Chen, and M. G. Lee, *Res. Commun. Chem. Pathol. Pharmacol.*, **32**, 27 (1981).

(2) M. A. F. Gadalla, G. W. Peng, and W. L. Chiou, *J. Pharm. Sci.*, **67**, 869 (1978).

(3) C. Brun, T. Hilden, and R. Raaschou, *J. Clin. Invest.*, **28**, 144 (1949).

(4) W. L. Chiou and G. Lam, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, in press.

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[1] Sigma Chemical Co., St. Louis, Mo.
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[3] Model 341, Sage Instruments, Cambridge, Mass.