This striking difference between similarly designated columns may represent the possible extremes in retention. Columns should be tested for suitability prior to use for the official method (1). Alternatively, either the internal standard can be omitted (3) if a precision loop injector[5] is used or the mobile phase composition may be altered to change the elution order of the steroids.

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[5] Rheodyne model 7010 or equivalent.

Effect of Plasma Protein Binding on Renal Clearance of Drugs

Keyphrases □ Plasma protein binding—effect on renal clearance of drugs □ Drug clearance, renal—effect of plasma protein binding □ Renal clearance—effect of plasma protein binding

To the Editor:

The theoretical concepts of the relationships between plasma or serum protein binding and hepatic metabolic clearance of drugs are now reasonably well defined (1–3 and references cited therein). On the other hand, corresponding theory and experimental data concerning the effect of plasma protein binding on the renal excretion of drugs are quite limited (4–13). The purpose of this communication is to propose certain relationships between plasma protein binding and renal clearance of drugs that may be useful for the design and interpretation of experimental studies.

The renal excretion of drugs usually involves three processes: glomerular filtration, renal tubular secretion, and partial reabsorption from the renal tubular lumen. Glomerular filtration is a passive process and may be assumed to be a function of the free (unbound) concentration of drug in plasma (11) if the glomeruli are intact. Renal tubular secretion is a specialized process; it is saturable in principle but appears to be linear for most drugs under the usual clinical or experimental conditions. The rate of renal tubular secretion may be proportional to the concentration of free or total (free and bound) drug in plasma; it may or may not be affected by blood flow. Renal tubular reabsorption of most drugs involves passive diffusion of nonionized molecules from the renal tubular lumen. Therefore, the rate of reabsorption is proportional to the concentration gradient of diffusible (usually free and nonionized) drug across the renal tubular boundary. Consequently, reabsorption may be affected by the urine flow rate and by the urine pH if the drug is a weak acid or base. The concentration of diffusible drug on the tissue side of the renal tubule is likely to be negligible compared to that in the lumen of the tubule, except in some cases of pronounced diuresis or urine pH alteration. Drug in the urine exists in unbound form unless there is serious nephropathy with marked proteinuria[1]. Thus, if the glomerular excretion rate is proportional to and the renal tubular secretion rate is a function of the concentration of *free* drug in plasma:

$$\text{renal excretion rate} = k_g fC + \frac{Qfk_sC}{Q + fk_s} - F\left(k_g fC + \frac{Qfk_sC}{Q + fk_s}\right) \quad \text{(Eq. 1)}$$

where k_g is the glomerular filtration clearance and k_s is the intrinsic renal tubular secretion clearance (both clearances are referenced to the free drug concentration in plasma), Q is the flow rate of plasma perfusing the renal tubular secretion sites, f is the free fraction of drug in plasma (which is assumed to be independent of concentration in the usual therapeutic or experimental concentration range), C is the concentration of total drug in plasma, and F is a (possibly urine flow rate- and urine pH-dependent) dimensionless constant equal to the fraction of filtered and secreted drug that is reabsorbed.

Implied in the equation is the assumption that F for filtered and secreted drug is the same; this assumption is reasonable if secretion takes place in the proximal region of the tubules and reabsorption occurs mainly from the distal region of the renal tubules. If the concentration ratio, erythrocytes:plasma, of the drug is substantial and reequilibration of the drug between erythrocytes and plasma is very rapid, C may be designated as the concentration of drug in whole blood and k_g, k_s, Q, and f have to be defined accordingly. However, this approach may be complicated if, as was suggested (9), a proportion of the erythrocytes is separated off by "plasma skimming" and shunted into the renal veins without contacting the renal tubules.

The second term on the right side of Eq. 1 is analogous to, and derived in a similar manner as, the hepatic metabolic clearance equation (2). If $Q \gg fk_s$, that term reduces to fk_sC and Eq. 1 reduces to:

$$\text{renal excretion rate} = k_g fC + k_s fC - F(k_g fC + k_s fC) \quad \text{(Eq. 2)}$$

Since renal clearance equals excretion rate/C, division of both sides of Eq. 2 by C and rearrangement yield:

$$\text{renal clearance} = f[k_g + k_s - F(k_g + k_s)] \quad \text{(Eq. 3)}$$

Therefore, a plot of renal clearance *versus* f should be linear and intersect the origin. This situation appears to be the case with salicylic acid in rats, according to preliminary results obtained in this laboratory[2]. If tubular reabsorption is prevented (which can be done with certain weak acids or bases by changing the urine pH), $F = 0$ and the slope of a plot of renal clearance *versus* f increases to $(k_g + k_s)$. The value of $(k_g + k_s)$ should not exceed the renal blood flow unless the compound is formed entirely or in part in the kidneys.

[1] Another rare exception is the case in which a drug or endogenous substance and a complexing or chelating agent are excreted concurrently, separately and as the complex. Each of these species will exhibit distinct renal pharmacokinetic characteristics.
[2] To be published.

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If tubular secretion is blocked ($k_s = 0$) and k_g is assumed to equal the glomerular filtration rate (GFR), Eq. 3 becomes:

$$F = 1 - (\text{renal clearance}/f\ \text{GFR}) \quad \text{(Eq. 4)}$$

A similar form of that equation was developed by Arita *et al.* (14); the inverse of the term in parentheses is the excretion ratio as defined by Fisher *et al.* (15). With F determined by means of Eq. 4, k_s can be calculated by a rearranged form of Eq. 3:

$$k_s = \frac{\text{renal clearance}}{f(1 - F)} - \text{GFR} \quad \text{(Eq. 5)}$$

It is reasonable to assume that k_g, being referenced to free drug and characterizing a physical rather than enzymatic or carrier process, is equal to the glomerular filtration rate, a constant that can be determined from the renal clearance of inulin or creatinine.

If the rate of renal tubular secretion is proportional to the concentration of *total* drug in plasma and if the previously stated assumptions apply:

$$\text{renal excretion rate} = k_g fC + k_s^* C - F(k_g fC + k_s^* C) \quad \text{(Eq. 6)}$$

where k_s^* is the renal secretion clearance referenced to the total drug concentration in plasma. Division of both sides of the equation by C and rearrangement yield:

$$\text{renal clearance} = fk_g(1 - F) + k_s^*(1 - F) \quad \text{(Eq. 7)}$$

In this case, a plot of renal clearance *versus* f should be linear and have a positive intercept. Such a relationship was observed recently by Yacobi and Levy (7) with respect to the renal clearance of sulfisoxazole in rats.

Dividing the slope of a plot of renal clearance *versus* f according to Eq. 7 by its intercept gives:

$$\frac{\text{slope}}{\text{intercept}} = \frac{k_g(1 - F)}{k_s^*(1 - F)} = \frac{k_g}{k_s^*} \quad \text{(Eq. 8)}$$

Consequently, inhibition of renal tubular secretion (for example, by administration of probenecid in the case of certain acidic drugs) should cause an increase of the slope:intercept ratio if the renal tubular secretion rate is proportional to the total drug concentration in plasma.

Inhibition or absence of renal tubular reabsorption ($F = 0$) reduces Eq. 7 to:

$$\text{renal clearance} = fk_g + k_s^* \quad \text{(Eq. 9)}$$

and, therefore, results in increased slope and intercept values but has no effect on the slope:intercept ratio. The slope should not exceed the glomerular filtration rate of a completely filtered substance such as inulin or creatinine, and k_s^* should not exceed the renal blood flow minus k_g unless the excreted substance is entirely or partly formed in the kidneys.

Equation 8 may be useful for estimating k_s^*, again with the assumption that k_g = GFR. Then, k_s^* = GFR (intercept/slope). Thus, it may be possible to estimate k_s^* under physiological conditions, *i.e.*, without changing the urine pH to prevent renal tubular reabsorption or administering a drug that blocks renal tubular secretion. Since the intercept of a plot of renal clearance *versus* f according to Eq. 7 is equal to $k_s^*(1 - F)$, F can be estimated if k_s^* is known.

Proportionality between the renal tubular secretion rate and the total rather than the free drug concentration in plasma may be a consequence of blood flow rate-limited secretion (12). Under these conditions, the sum of k_g and k_s^* may be equal to the renal blood flow or it may be less than that if part of the blood perfusing the kidneys bypasses the sites of renal secretion for a particular drug (12, 16). Thus, Eq. 6 may be a limiting case of Eq. 1 when $fk_s \gg Q$. Then the second term on the right side of Eq. 1 reduces to QC, and k_s^* in Eqs. 6–9 becomes Q.

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