tion of these endogenously produced inhibitors is not established definitively, their activity in plasma was determined indirectly by measuring their effect on the protein binding of a suitable marker substance, bilirubin.

Intravenous injection of heparin causes a decrease in the plasma protein binding of bilirubin and certain drugs, apparently due to increased concentrations of fatty acids (7–9). In this study, male adult Sprague–Dawley rats had a cannula inserted in the femoral vein under ether anesthesia. A blood sample (~0.7 ml) was then collected. Immediately thereafter, the rats received an intravenous injection of heparin (500 units/kg) or an equal volume of normal saline solution. The liver was then exposed through a midline abdominal incision, a cannula was inserted in the hepatic vein of the left lobe (10), and the hepatic vein was clamped near its junction with the vena cava immediately before the hepatic vein blood sample was taken. These procedures were completed within 1–3 min.

Blood samples (0.7 ml) were obtained simultaneously from the femoral and hepatic veins from 3 to 5 min after heparin injection. Plasma was separated, bilirubin was added to yield a final concentration of ~10 mg/100 ml, and the free fraction of this substance was determined by a peroxidase-catalyzed reaction rate method (3, 11). Blood samples obtained before heparin or saline injection were treated in the same manner.

In the control (saline injection) experiments, the plasma bilirubin free fraction values (mean $\times 10^4 \pm SD$, $n = 6$) were 3.06 ± 0.63 before injection, 3.75 ± 1.05 in plasma from the femoral vein after injection, and 3.98 ± 0.87 in plasma from the hepatic vein after injection. The ratio of the bilirubin free fraction values, hepatic vein plasma: femoral vein plasma, was 1.08 ± 0.10 (mean ± SD, $n = 6$). Thus, perfusion through the liver had no apparent effect on the bilirubin binding characteristics of plasma in control animals.

Heparin injection caused a rapid and pronounced increase in the plasma free fraction of bilirubin as reported previously (7). Moreover, there was a pronounced difference between the protein binding of bilirubin in femoral vein and hepatic vein plasma. The ratio of the bilirubin free fraction values, hepatic vein plasma:femoral vein plasma, was 0.647 ± 0.192 (mean ± SD, $n = 11$).

The results of this investigation demonstrate that certain endogenous inhibitors of plasma protein binding are extracted quite efficiently by the liver. The plasma protein binding of drugs affected by these inhibitors is not the same in plasma obtained from a peripheral vein and in plasma obtained immediately after passage through the liver. Therefore, estimations of the intrinsic clearance of free drug in the presence of inhibitors of protein binding of that drug are incorrect if these inhibitors have large hepatic (or other eliminating organ) extraction ratios.

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High-Pressure Liquid Chromatography of Triamcinolone Acetonide: Effect of Different Octadecylsilane Columns on Mobility

Keyphrases □ Triamcinolone acetonide—high-pressure liquid chromatographic analysis, effects of various octadecylsilane columns on mobility □ High-pressure liquid chromatography—analysis, triamcinolone acetonide, effects of various octadecylsilane columns on mobility □ Anti-inflammatory agents—triamcinolone acetonide, high-pressure liquid chromatographic analysis, effects of various octadecylsilane columns on mobility

To the Editor:

Triamcinolone acetonide[1], a topical anti-inflammatory agent, was assayed by high-pressure liquid chromatography (1) with fluoxymesterone as the internal standard. A reversed-phase octadecylsilane column was used with a mobile phase of acetonitrile–water (30:70) at a flow rate of 2 ml/min. Detection was at 254 nm.

With a column[2] packed with particles 10% covered by octadecylsilane, fluoxymesterone eluted in 10 min and triamcinolone acetonide eluted in 12 min. With a column[3] covered with 5% octadecylsilane, and under otherwise identical chromatographic conditions, fluoxymesterone eluted in 8 min and triamcinolone acetonide eluted in 6 min. The elution patterns were reversed, as also was shown by separate injections of the individual steroids and linearity studies using various concentrations of both steroids.

Both columns are ostensibly the same L-1 type (2). This reversal may be due to the difference in coverage of the particles by octadecylsilane[4] if no other proprietary differences are assumed. The former column separates primarily on the basis of partition, and the latter separates primarily by a combination of partition and adsorption. [The order of elution using the first column can be reversed by using a mobile phase of methanol–water (60:40) instead of acetonitrile–water (30:70).] Both columns gave similar values for triamcinolone acetonide.

[1] Kenalog, E. R. Squibb.
[2] μBondapak, Waters Associates.
[3] Partisil, Whatman.
[4] Manufacturers' literature.

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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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