Supported by funds from the John R. and Marie K. Sawyer Memorial Fund, Massachusetts College of Pharmacy.

The authors are indebted to Dr. Ralph G. Child, Lederle Laboratories, and to the Division of Cancer Treatment, National Cancer Institute, for the antileukemia test results, and to Dr. Ram Ganapathi, University of Miami School of Medicine, for carrying out the cell culture studies.

COMMUNICATIONS

Hepatic Extraction of Endogenous Inhibitors of Plasma Protein Binding

Keyphrases □ Drug binding—plasma protein, hepatic extraction of endogenous inhibitors □ Pharmacokinetics—plasma protein binding, hepatic extraction of endogenous inhibitors □ Plasma protein binding—hepatic extraction of endogenous inhibitors

To the Editor:

Determination of the systemic intrinsic clearance of free (unbound) drug requires measurement of the drug's free fraction in blood, plasma, or serum. For drugs whose total clearance from the blood is much lower than the blood flow through the eliminating organ(s), the intrinsic clearance of the free drug is equal to the total clearance divided by the free fraction (1, 2). The use of free fraction values obtained in the usual manner (*i.e.*, by *in vitro* determinations of protein binding in plasma from peripheral venous blood) for intrinsic clearance calculations is based on the assumption that drug protein binding does not change as the blood passes through the liver or another drug-eliminating organ.

The free fraction value of many drugs in blood or plasma is reasonably constant over a wide drug concentration range. Consequently, it will not be affected by modest drug concentration changes during a single pass of blood through the liver. If, however, protein binding of the drug is affected by endogenous or exogenous (other drugs) inhibitors of binding, then the organ extraction ratio of these inhibitors must be considered. The potential consequences of hepatic extraction of binding inhibitors on the pharmacokinetics and pharmacological activity of a restrictively cleared drug can be appreciated by considering the following limiting cases.

If the extraction ratio [(concentration in inflowing blood − concentration in outflowing blood)/concentration in inflowing blood] of inhibitors of protein binding is close to zero (*i.e.*, there is very little extraction), then the inhibitors will cause an increase in the free fraction, a decrease in the steady-state total concentration, and *no change* in the steady-state free concentration of drug in peripheral blood or plasma (3). Since the pharmacological activity of a drug is usually a function of its free concentration (4), the intensity of pharmacological activity at steady state should not be affected by poorly extracted inhibitors of binding unless they have pharmacological effects of their own.

Conversely, if an inhibitor of protein binding is very rapidly and completely extracted from blood by the liver during a single pass (extraction ratio is near unity and the time for extraction of inhibitor from blood is much shorter than the transit time of that blood through the liver), then the inhibitor will have little or no effect on the steady-state concentration of total (free plus bound) drug, but it will cause the free fraction and the steady-state concentration of free drug in extrahepatic blood or plasma to be *increased*. This result would come about for two reasons:

1. Protein binding of the drug in blood is normal (rather than reduced) during most of the residence time of any one small portion of blood in the liver.
2. Protein binding of the drug leaving the liver is soon reduced as that blood mixes with inhibitor-containing blood in the vena cava, causing the free fraction and concentration of free drug to rise again.

Drug binding inhibitors with hepatic extraction ratios near unity may be cleared quite rapidly from the body. However, their concentration in blood can be quite stable if they are continuously formed endogenously, are continuously administered (other drugs or dietary components), or have a large apparent volume of distribution. Such inhibitors can be expected to increase the pharmacological activity of drugs that they displace by increasing the steady-state free concentration of these drug in extrahepatic blood. Since the steady-state concentration of total drug may be almost unchanged, the increased pharmacological activity may be unanticipated.

It is relatively easy to determine the hepatic extraction characteristics of drugs that act as displacers of other drugs from plasma protein binding sites; such a determination can be done by measuring the hepatic clearance of the displacer drugs. However, this approach is not possible (at least at this time) in the case of endogenous inhibitors associated with certain changes in the pathophysiological status of humans and animals. The hepatic extraction characteristics of binding inhibitors associated with pregnancy (5) and renal failure (6) are presently being investigated directly. To describe the method used for this investigation and to demonstrate in principle that certain endogenous inhibitors are indeed subject to considerable hepatic extraction, data on heparin-induced inhibitors of protein binding will be reported here. Since the composi-

0022-3549/80/0400-0480$01.00/0

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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Received October 22, 1979.
Accepted for publication January 4, 1980.
Supported in part by Grant GM 19568 from the National Institutes of Health.
The technical assistance of Mr. David M. Soda is gratefully acknowledged.

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.

0022-3549/80/0400-0481$01.00/0