the same in the reference and test studies when the total amount excreted unchanged in the urine is used for determination of bioavailability.

A number of drugs, however, demonstrate urine pH and urine flow dependent renal clearance; unless the experimental conditions are rigidly controlled by acidifying or alkalinizing the urine pH with ammonium chloride or sodium bicarbonate while maintaining constant fluid intake, renal plasma clearance, Cl_R, is likely to vary between and within test and reference studies.

Kwan and Till (7) suggested a method to circumvent this situation. When renal plasma clearance differs between studies, the renal plasma clearance can be determined from the renal excretion rates and the plasma concentrations of the unchanged drug between the experiments. The assumptions in this method are that renal clearance is constant within an experiment (but, of course, can vary between studies) and that the extrarenal plasma clearance is the same between studies.

The assumption of constant renal plasma clearance throughout the individual studies is, at best, an approximation. When studies have to be carried out over longer periods, the renal clearance may vary due to fluctuations in urine flow and urine pH throughout the day, providing the renal clearance is urine pH and urine flow dependent.

We propose a method for the determination of bioavailability that does not require constant renal clearance, neither between experiments nor within an experiment. This method allows for the determination of the bioavailability of a drug without rigid control of urine pH or urine flow, provided the extrarenal plasma clearance is unaltered.

The elimination rate, dA_e/dt, can be described by:

$$\frac{dA_e}{dt} = \frac{dA_{xr}}{dt} + \frac{dA_r}{dt} \qquad \text{(Eq. 1)}$$

where dA_{xr}/dt is the rate of extrarenal elimination and dA_r/dt is the rate of renal excretion.

The extrarenal elimination can be expressed by:

$$\frac{dA_{xr}}{dt} = (Cl_{xr})(C_p) \qquad \text{(Eq. 2)}$$

where Cl_{xr} is the extrarenal clearance and C_p is the plasma concentration.

Substitution of Eq. 2 in Eq. 1 and rearrangement give:

$$dA_e = (Cl_{xr})(C_p)(dt) + dA_r \qquad \text{(Eq. 3)}$$

The total amount eliminated from the body from time = 0 to time = ∞ must be equal to the total amount entering the general circulation, *FD, and* the total amount excreted in the urine from time = 0 to time = ∞ must be equal to the total amount recovered in the urine, A_R^∞.

Therefore, integrating Eq. 3 from time = 0 to time = ∞ gives:

$$FD = \int_0^\infty (Cl_{xr})(C_p)(dt) + A_R^\infty \qquad \text{(Eq. 4)}$$

where F is the bioavailability of the dosage form and D is the amount of drug administered.

Since $\int_0^\infty (C_p)(dt) = AUC_0^\infty$ and Cl_{xr} is assumed to be a constant, the following relationship is obtained:

$$FD = (Cl_{xr})(AUC_0^\infty) + A_R^\infty \qquad \text{(Eq. 5)}$$

When the bioavailability of the reference dose is F^s and the bioavailability of the test dosage form is F^T, F^T can simply be determined by:

$$F^T = \frac{(F^sD^s - A_R^{\infty s})\dfrac{AUC_0^{\infty T}}{AUC_0^{\infty s}} + A_R^{\infty T}}{D^T} \qquad \text{(Eq. 6)}$$

When the reference dose is an intravenous bolus dose where the bioavailability is equal to 1, Eq. 6 can be expressed by:

$$F^T = \frac{(D^s - A_R^{\infty s})\dfrac{AUC_0^{\infty T}}{AUC_0^{\infty s}} + A_R^{\infty T}}{D^T} \qquad \text{(Eq. 7)}$$

To use this method, the total area under the plasma concentration *versus* time curve and the amount excreted unchanged in the urine from time = 0 to time = ∞ for both the test drug and the standard are needed. Except for the assumption of extrarenal plasma clearance constancy, this method does not require a constant renal clearance and fraction excreted unchanged nor an assessment of the pharmacokinetic model. From Eq. 6, it can be seen that this method cannot be used to assess the relative bioavailability of a drug, F^T/F^s, and is only useful when the reference dosage form is an intravenous bolus or if the bioavailability of the reference dose is known.

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Novel Method for Determining Protein Binding of Theophylline

Keyphrases □ Theophylline—protein binding determined by ultrafiltration method □ Protein binding—theophylline, determined by ultrafiltration method □ Binding, protein—theophylline, determined by ultrafiltration method □ Ultrafiltration method—determination of theophylline protein binding □ Relaxants, smooth muscle—theophylline, protein binding determined by ultrafiltration method

To the Editor:

The kinetics of drug elimination and the apparent biological half-life of a drug may be influenced by drug–protein interactions (1). Although interpatient and intrapatient variations in theophylline clearance rates are gen-

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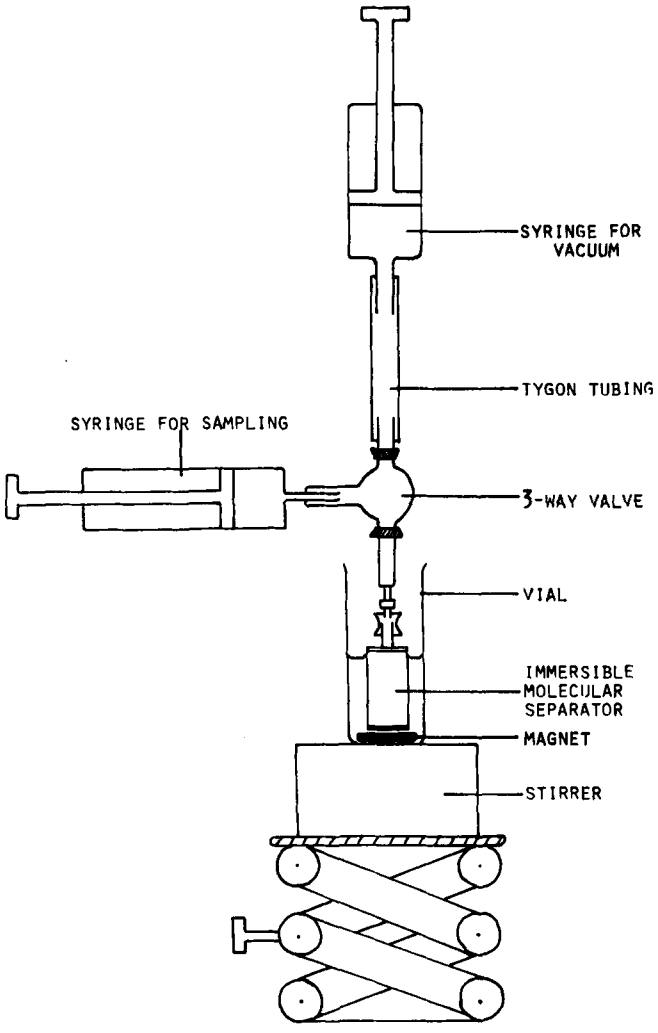

Figure 1—*Ultrafiltration apparatus using the immersible membrane cartridge.*

erally attributed to factors such as age, smoking, ingestion of other drugs, and hepatic dysfunction (2), protein binding of theophylline also could influence clearance.

Theophylline binding to bovine serum albumin (fraction V) (3) and human plasma (4) has been determined by equilibrium dialysis, but this technique is slow and unsuitable for routine clinical use. Conventional ultrafiltration is more rapid and has been used to study the binding of theophylline to serum and human milk (5). Ultrafiltration cones have been employed to measure protein binding of theophylline in small (2 ml) plasma samples (6). A controlled-temperature centrifuge is required for reproducible results with these ultrafiltration techniques.

The extent of theophylline binding to human serum and bovine serum albumin (fraction V) was determined using an ultrafiltration method that eliminates centrifugation. An immersible cartridge[1] was used, consisting of a noncellulosic ultrafiltration membrane sealed to a plastic core. Each cartridge was connected to a 3-ml disposable syringe *via* a three-way valve (Fig. 1). The syringe provided a sufficient vacuum to effect ultrafiltration when the cartridge was immersed in the test material. The ultrafiltrate was sampled using the second syringe.

The human serum and bovine serum albumin solution (4%) in phosphate buffer, pH 7.4, contained 15 μg of theophylline/ml. The immersible cartridge unit was flushed with pH 7.4 buffer prior to use, and excess buffer was removed by suction. The cartridge was immersed in 2–4 ml of the theophylline–protein specimen to be analyzed, and the sample was agitated gently with a magnetic stirring bar while avoiding physical damage to the separator membrane. A vacuum was applied to the core until sufficient protein-free filtrate was obtained. The aliquot collected in protein-binding studies by ultrafiltration did not exceed 10% of the initial volume. Samples of the filtrate and retentate were removed and assayed for theophylline using high-performance liquid chromatography (7). The extent of binding was calculated using:

$$\text{fraction bound} = \frac{D_b}{D_t} = \frac{D_t - D_f}{D_t} \qquad \text{(Eq. 1)}$$

where D_b is the amount of drug bound, D_t is the total amount of drug present, and D_f is the amount of free drug. Each immersible cartridge unit was reused several times after careful washing according to the procedure recommended by the manufacturer.

The immersible cartridge and ultrafiltration cone[2] methods were compared at room temperature. The cones were soaked in pH 7.4 phosphate buffer, removed, and wiped dry. Protein solution or serum containing theophylline, 2–4 ml, was placed in the cone and centrifuged at 800×*g*. The samples were spun three times (2–6 min each) to provide three 100-μl aliquots. The second and third aliquots were assayed for theophylline to give the concentration of free drug in the original sample. The solution in the cone was analyzed for total concentration of theophylline.

Both ultrafiltration cones and immersible cartridge units permitted protein leakage into the filtrate if the membrane was not functioning correctly or was damaged. Such leakage would invalidate results since the protein transports bound theophylline. A sensitive colorimetric semiquantitative estimate for protein[3] was used to test all filtrates.

The percentage binding of theophylline at concentrations of 15 μg/ml to serum or bovine serum albumin (fraction V) was similar when determined by the immersible cartridge and ultrafiltration cone methods. With the 4% bovine serum albumin, theophylline protein binding was 56.3 ± 7.4% using the immersible cartridge and 51.7 ± 11.4% using the ultrafiltration cones. In pooled human serums, theophylline protein binding was 67.8 ± 5.5% using the immersible cartridge and 66.3 ± 4.6% using the ultrafiltration cones. The results obtained from the immersible cartridge and cones were not significantly different ($p < 0.05$).

Determination of theophylline protein binding using immersible cartridges is convenient and rapid. The ultrafiltration membrane has a nominal molecular weight cutoff of 10,000, which provides effective separation of

[1] Immersible molecular separator, Millipore Ltd., Mississauga, Ontario, L4V 1L2, Canada.

[2] Centriflo CF 50-A cones, Amicon Corp., Lexington, MA 02173.

[3] Albustix, Ames Co. Division, Miles Laboratories, Ltd., Rexdale, Ontario, Canada.

theophylline from serum or plasma proteins. The apparatus is portable and does not require a refrigerated centrifuge for ultrafiltration. The procedure is ideal for clinical investigations because of the small volume of sample required. It has potential application to the study of protein binding of a wide range of xenobiotic compounds.

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BOOKS

REVIEWS

Formulation and Preparation of Dosage Forms. Edited by J. POLDERMAN. Elsevier/North-Holland, 52 Vanderbilt Ave., New York, NY 10017. 1977. 307 pp. 16 × 24 cm. Price $37.95.

This volume presents the proceedings of the 37th International Congress of Pharmaceutical Sciences (F.I.P.) held in The Hague, The Netherlands, September 5–9, 1977. The editor states that the book takes its title from the main theme of the Congress.

The first of five sections is Formulation and Preparation of Dosage Forms. Three reviews are given on formulation factors affecting drugs given by oral route, surface applied drugs, and drugs given parenterally. These reviews are preceded by a General Introduction and followed by Conclusions and Perspectives. The introduction was more informative and better documented than the presentations on the oral route and the parenteral route.

The second section is Drug Substance-Pro-Drugs. The presentation, Drug Substances in Particular Pro-Drugs: Problems and Methods of Approach, was excellent and provided a wealth of references. The report, Analytical Aspects on Pro-Drugs, was informative and well documented. Pro-Drugs: Structure Activity Relationships suffers by comparison with the other presentations.

The third section, First Pass Effects, is composed of three lectures covering the influence of the route of administration of a substance on its bioavailability, drug metabolism associated with the routes of administration, and first pass effects and consequences for the routes of administration and dosage form design. The section describes the first pass effects and cites examples; however, some statements are redundant. Perhaps this repetition is unavoidable with the multiauthor presentations of a symposium.

The fourth section, Mechanism of Drug Release, consists of three presentations. Physico-chemical Aspects of Drug Release discusses theories of dissolution and dissolution of particles and binary mixtures. Solid Dosage Forms: Mechanism of Drug Release gives a simple view of the effect of formulation on release. Liberation of Medicaments from Semi-solid Bodies Applied to the Skin considers penetration conditions of chemical substances through the skin and the role of carrier materials in the rate of release of medicaments applied to the skin.

The final section, Physico-chemical and Technological Aspects, was also composed of three presentations. Powder technology was discussed superficially. A description of physicochemical and technological aspects of granulation techniques was elemental. A review of the process involved in tablet formulation provided some interesting scanning electron micrographs of compressed tablets; however, it did not explain or document what might be occurring in the compaction process. The final section was the least informative of the five sections.

As expected in a symposium presented by 17 authors in three languages, even after translation into English, the style is not uniform and repetition does occur; however, the volume is easily read and provides facts and references of interest to anyone concerned with dosage forms.

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Cardiovascular Drugs. Vol. 1: Antiarrhythmic, Antihypertensive and Lipid Lowering Drugs. Vol. 2: β-Adrenoceptor Blocking Drugs. Edited by GRAEME S. AVERY. University Park Press, 233 East Redwood St., Baltimore, MD 21202. 1978. 16 × 24 cm. Vol. 1: 176 pp. Vol. 2: 230 pp.

These two volumes comprise two parts of a three-part series entitled "Cardiovascular Drugs." The chapters have been revised from popular review articles previously published in the Australasian Drug Information Services press journal, *Drugs*. Written by internationally recognized authorities in their respective fields, this collected series provides a concise and convenient review of the current state of the art in cardiovascular drug therapy.

Volume 1 contains four chapters that review lipid lowering drugs and hyperlipidemia, antihypertensive drug therapy, antiarrhythmic agents, and clinical pharmacology and therapeutic uses of digitalis glycosides. With 51 figures, 19 tables, over 500 references, and an extensive subject index, this volume provides valuable and practical information concerning the appropriate therapeutic use of antiarrhythmic, hypotensive, and hypolipidemic drugs. Also included are discussions concerning the pharmacological actions, adverse reactions, and combination drug interactions of these cardiovascular agents.

Volume 2 contains nine chapters covering the pharmacodynamics and pharmacokinetics of β-adrenoreceptor blocking drugs; β-adrenoceptor blocking agents in the treatment of hypertension, angina pectoris, cardiac arrhythmias, and hyperthyroidism; clinical toxicology of propranolol and practolol; adverse effects of β-adrenoceptor blocking agents on respiration; and autoimmune and autoallergy phenomena in patients treated with β-blockers. With 20 figures, 27 tables, over 1000 references, and a subject index, this book provides a comprehensive review of β-adrenergic blocking drugs.

These well-written and comprehensive volumes are excellent reference sources and clinical guides to scientists working in cardiovascular re-