Binding Rate of Antibacterials to Serum Proteins

Keyphrases □ Cefazolin—rate and extent of binding to serum protein, dogs □ Protein binding, serum—cefazolin, rate and extent, dogs □ Binding, serum protein—cefazolin, rate and extent, dogs □ Antibacterials—cefazolin, rate and extent of binding to serum protein, dogs

To the Editor:

It is generally accepted that the equilibrium between protein and drug occurs instantaneously, *i.e.*, within a fraction of a second (1). Measurement of the time necessary for the equilibrium between levothyroxine and serum protein showed that equilibrium was reached within 150 msec (2). Other reports confirmed the rapidity of this binding reaction (3, 4).

Contrary to previously published reports, Waterman *et al.* (5) reported that up to 50 min was required to attain the equilibrium between dog serum and several cephalosporin antibacterials. Cefazolin was approximately 20% protein bound to dog serum at 10 min after mixing and 80% bound after 50 min. This reported (5) value for the maximum binding of cefazolin (80%) to canine serum proteins was vastly different from that reported by Nishida *et al.* (6). They found 20% 1 hr after mixing of the drug–protein solution, but they did not measure binding as a function of time.

If these results are truly representative of the time necessary for the attainment of the protein-binding equilibrium, the effect of this lag time on drug distribution and elimination would be extremely complex. The cephalosporin antibacterials have a distributive phase with a half-life of 10 min (7). If it takes 50 min to reach the protein-binding equilibrium, then the drug will have distributed before maximum binding is attained. This result would have a drastic effect on the tissue availability of unbound drugs. Due to the pharmacokinetic and possible clinical implications involved with prolonged rates of attainment of the protein-binding equilibrium, we decided to investigate the rate and extent of the binding of cefazolin to serum protein.

Five milliliters of a 200-μg/ml solution of cefazolin in canine serum was added to 45 ml of canine serum at 37° to yield a serum cefazolin concentration of 20 μg/ml. Time zero was defined as the time the cefazolin was introduced into the flask containing the serum. The solution was stirred by a magnetic stirrer. At selected times during the hour after mixing, 7-ml samples of the cefazolin–serum solution were removed from the stirred flask and placed in the ultrafiltration apparatus[1] having an ultrafiltration membrane with a molecular weight cutoff of 10,000[2].

Nitrogen gas (12 psi) was applied to the system, and the protein-free filtrate was collected. The first 0.4 ml of filtrate samples was discarded since this portion was required to saturate all drug binding sites on the membrane. The next 0.6 ml was collected, and the time of collection was noted. All filtrates were frozen until assayed microbiologically using a disk diffusion assay with *Bacillus subtilis* as the test organism (8).

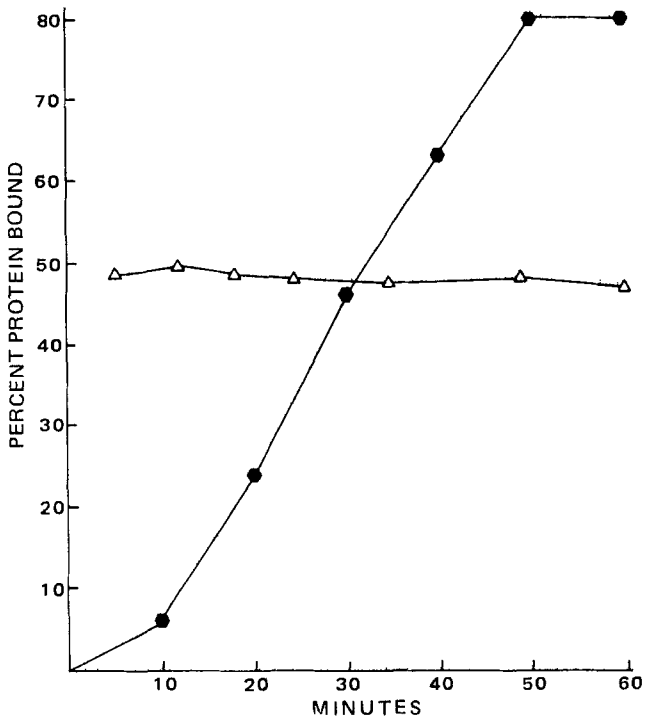

Figure 1—*Cefazolin binding to canine serum. Samples were taken as a function of time after mixing of serum and cefazolin. Total cefazolin concentration was 20 μg/ml. Key: △, data from the present study; and ●, data from Ref. 5.*

All experiments showed that the binding equilibrium was attained within 3–5 min, the shortest possible measurement time with the ultrafiltration apparatus. Approximately 48% of the total drug was in the protein-bound form throughout the hour interval of the experiment (Fig. 1), which is higher than the previously reported value for the binding of cefazolin to canine serum (6) but significantly lower than the value reported by Waterman *et al.* (5). No changes in binding were found with canine serum as a function of time. The previously reported (5) prolonged time to reach the protein-binding equilibrium was probably artifactual due to a technical problem with ultrafiltration.

Since a considerable species difference exists between the binding capacity of dog serum (48%) *versus* human serum (80%) (7), caution is advised in extrapolating experiments with a dog model to the human situation unless it has been previously established that the degree and extent of protein binding to serum protein are similar. This is especially true of comparative studies involving tissue or interstitial fluid distribution of drugs that are highly protein bound. For example, a comparison of cephaloridine, cephradine, or cephalexin (all poorly bound) to cefazolin might show equivalent distribution patterns in the interstitial fluid of dogs (9) but vastly different pat-

[1] Amicon MMC, Lexington, Mass.
[2] Amicon PM-10, Lexington, Mass.

terns in humans. Although other animal species represent convenient models for studying interstitial fluid distribution patterns of antibacterials, we feel that a significant potential for misinterpretation of the data exists unless the protein-binding characteristics of the antibacterial in the animal species are similar to those of humans.

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D. S. Greene *
R. Quintiliani
C. H. Nightingale ˣ
School of Pharmacy
University of Connecticut
Storrs, CT 06268
and
Department of Medicine
Hartford Hospital
Hartford, CT 06115

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* Present address: College of Pharmacy, University of Rhode Island, Kingston, RI 02881.
ˣ To whom inquiries should be directed. Present address: School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.

BOOKS

REVIEWS

Drug Fate and Metabolism: Methods and Techniques, Vol. 1. Edited by EDWARD R. GARRETT and JEAN L. HIRTZ. Dekker, 270 Madison Ave., New York, NY 10016, 1977. 313 pp. 15 × 23 cm. Price $35.00.

This volume is the first of a proposed series of texts on drug fate and metabolism. As indicated by the editors, the intent of this volume and future volumes is "to review all the techniques, physical, chemical, biological, medical, and mathematical, which can be applied to the study of drug fate in the organism. It is addressed primarily to the research scientist and is devoted to *methods*, with only the minimal theory given for perspective, appreciation, and proper evaluation of results." Volume I meets these goals, with the major emphasis being placed on the use of analytical methods.

Chapters I and II deal with autoradiography and autoradiography in cytopharmacology. Both chapters were written by experts in their area of speciality and are of particular importance to a novice in autoradiography. Chapters III and IV explore the use of electrophoresis and ion-pair extraction and chromatography. Chapter V reviews protein binding, and Chapter VI briefly looks at atomic absorption spectroscopy. The use of a fairly recent analytical method, spin immunoassay, is discussed in Chapter VII. The last chapter describes facilities and other pertinent information needed in animal care.

The editors state that "it was deemed proper to include chapters on methods that would not be modern methods of choice but are of historical importance in evaluating the significance and limitations of the earlier studies in these fields." The selection of these methods is one notable weakness that occurs in Volume I. All of the volumes in this series apparently will be required if an individual desires to have the methods most frequently used in drug metabolism. Another weakness is that the chapters have no rhyme or reason to their order. It would be more sensible to have the chapter on animal care at the beginning of the book since metabolism studies are based on the use of animals as a testing source.

One strong feature of this text, in addition to its being well written, is a list of the source of supplies and equipment included in Chapters I and III. This list is particularly important in saving time that would otherwise be spent in looking for this material. The reviewer recommends this volume for the researcher in the area of drug metabolism, especially the analytical chemist, pharmacologist, and toxicologist.

Reviewed by Gerald W. Slywka
School of Pharmacy
Ferris State College
Big Rapids, MI 49307

Martindale: The Extra Pharmacopoeia. 27th Ed. Edited by AINLEY WADE and JAMES E. F. REYNOLDS. The Pharmaceutical Press, 1 Lambeth High Street, London, SE1 7JN, England, 1977. 19 × 25.4 cm. 2077 pp. Price $60. Available from Rittenhouse Book Distributors, 251 S. 24th St., Philadelphia, PA 19103.

The 27th edition of "Martindale" is the largest ever published. It has been revised thoroughly and updated and extended in coverage. The book is divided into six main sections. Part 1, the largest, contains monographs on more than 3100 drugs and ancillary substances in current use throughout the world. Drugs having similar uses or actions are grouped together. Part 2, Supplementary Drugs and Ancillary Substances, describes 1040 new drugs that are too recently introduced or of insufficient importance to be included in Part 1. Also in Part 2 are monographs on obsolescent drugs about which information may still be required.

Part 3 gives the composition and source of more than 1450 over-the-counter medicines marketed in Great Britain. Part 4 is a Directory of Manufacturers, listing full names and addresses of over 1400 manufacturers throughout the world. The Index to Clinical Uses, Part 5, is a guide to drug usage presented in alphabetical order of diseases.

The final section, the General Index, has more than 43,000 entries. Substances are indexed by generic names, proprietary names, chemical names, and synonyms.

This book represents the most comprehensive reference source of information on drugs used throughout the world. Pharmacists as well as professionals and students in all health-related and scientific fields will find this book most valuable. It is a necessity in every pharmacy and medical library.

Staff review