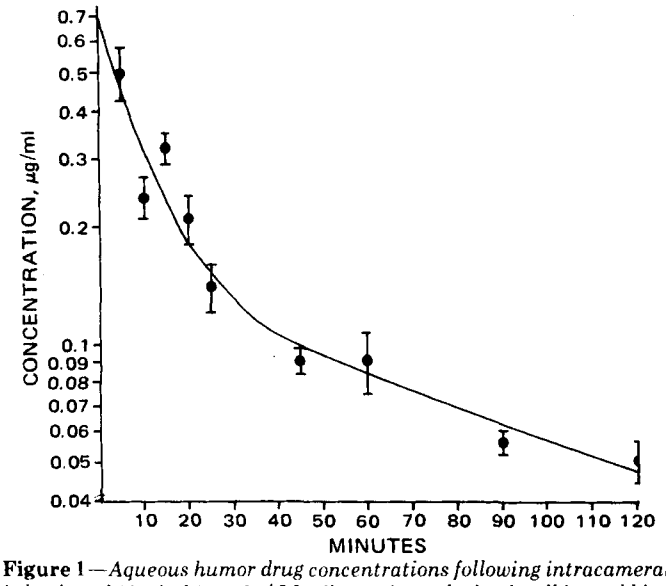

Figure 1—*Aqueous humor drug concentrations following intracameral injection of 10 μl of 1 × 10^{-4} M pilocarpine solution in albino rabbits. Points represent the mean of at least seven determinations. Bars represent the standard error of the mean. The solid line is the nonlinear regression line of the data fitted to a biexponential equation using the NONLIN program.*

absolute pilocarpine levels obtained are in reasonable agreement with those of Conrad and Robinson for the first 25 min. However, when later time points are considered, the curve clearly is not monoexponential. When the data reported here for the first 25 min are fitted to a monoexponential equation, an apparent volume of distribution and an elimination rate similar to those of Conrad and Robinson are obtained. However, due to the curvature noted at later time points, the short sampling period reported by Conrad and Robinson evidently resulted in an underestimation of the apparent ocular distribution volume for pilocarpine and represents a nonequilibrium apparent volume of distribution. This interpretation was mentioned by Conrad and Robinson but was rejected by reference to unpublished data.

The concept of a changing volume of distribution with time is, of course, not new and is expected with tissues that equilibrate slowly (7). Preliminary studies in this laboratory indicate that the partitioning of pilocarpine between aqueous humor and some ocular tissues is, in fact, a slow process. In addition, due to the effect of pilocarpine on aqueous humor turnover, it is to be expected that the slope of the pilocarpine decline from aqueous humor following intracameral injection would not remain constant over time. Ongoing experiments in this laboratory indicate that if pilocarpine levels are followed for times longer than those shown in Fig. 1, the slope becomes even more shallow. Therefore, a combination of factors, slow equilibration along with pilocarpine's pharmacological effect, makes it difficult to assign precise values to the ocular volume of distribution or, in turn, to the clearance of pilocarpine.

From a practical standpoint, apparent volumes of distribution obtained in short-term studies such as those of Conrad and Robinson may suffice for some drugs in describing aqueous humor drug levels in single-dose situations since most of the drug is eliminated quite rapidly. However, such studies usually will not suffice to predict aqueous humor drug levels over a long time or in steady-state situations. Therefore, pharmacokinetic data obtained during ocular multiple dosing or steady-state conditions may require a somewhat different interpretation than those obtained during short duration, single-dose studies.

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Applications of TLC and Bioautography to Detect Contaminated Antibiotic Residues: Tetracycline Identification Scheme

Keyphrases □ Cephalosporins—TLC–bioautographic analysis as contaminants in various tetracyclines □ Penicillin—TLC–bioautographic analysis as contaminant in erythromycin □ Tetracyclines, various—TLC–bioautographic analysis of four cephalosporins as contaminants □ Erythromycin—TLC–bioautographic analysis of penicillin and ampicillin as contaminants

To the Editor:

A method to detect penicillin and ampicillin as contaminants in various tetracyclines and penicillamine was reported previously (1). The method utilizes TLC followed by bioautography and has been applied to other contamination situations and antibiotic standard problems. The cephalosporins are related closely to penicillin in chemical structure, antimicrobial activity, and allergenicity. In addition to the occurrence of allergenic cross-reactivity with penicillin, several cases of apparent primary allergy to cephalosporins in patients not known to be hypersensitive have been reported (2). Minor modification of the assay permitted the separation of four cephalosporin contaminants from seven tetracyclines: demeclocycline, methacycline, minocycline, chlortetracycline, oxytetracycline, doxycycline, and tetracycline.

The original method was followed with several excep-

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Table I—R_f Values and Detection Limits of Cephalosporins

Antibiotic	R_f	Detection Limit, μg
Cephalothin	0.65	0.01
Cephaloridine	0.41	0.01
Cephalexin	0.59	0.005
Cephaloglycin	0.38	0.01

tions. The standard solutions of the cephalosporins were prepared at concentrations of 1.0, 0.5, 0.25, and 0.125 μg/ml, and the mobile phase composition was adjusted to a barbital acetate buffer (pH 7.4) to acetone ratio of 80:20 (v/v). The tetracyclines remained near the starting point; the cephalosporins migrated with the R_f values indicated in Table I.

Similarly, erythromycin is administered frequently to patients sensitive to penicillin. Thus, any contamination of erythromycin by penicillin is particularly undesirable for such patients. The existing conventional methods to detect penicillin in erythromycin are extraction procedures followed by cylinder–plate assay or direct cylinder–plate assay. These methods are time consuming and require much more sample handling than does this TLC method. Again, the same methodology was followed except that the mobile phase composition ratio was changed to 90:10 (v/v). Erythromycin base, erythromycin stearate, and erythromycin stearate tablets were assayed. The erythromycin remained at the spotting point, whereas the R_f value for penicillin G was 0.24 and that for ampicillin was 0.47.

The original system has been helpful in assessing the purity of some antibiotic standards. UV spectrophotometry is the official identity test for many antibiotics, including penicillin V. When a proposed standard of penicillin V potassium was assayed, the spectrum showed an additional peak. The following solutions were chromatographed in this system: penicillin V potassium, the proposed standard; penicillin V potassium, the working standard; a mixture of penicillin G potassium and penicillin V potassium; penicillin G potassium, the working standard; and *p*-hydroxypenicillin V potassium. The results are shown in Fig. 1. Carbenicillin, amoxicillin, neomycin, and cyclacillin were assayed using this system.

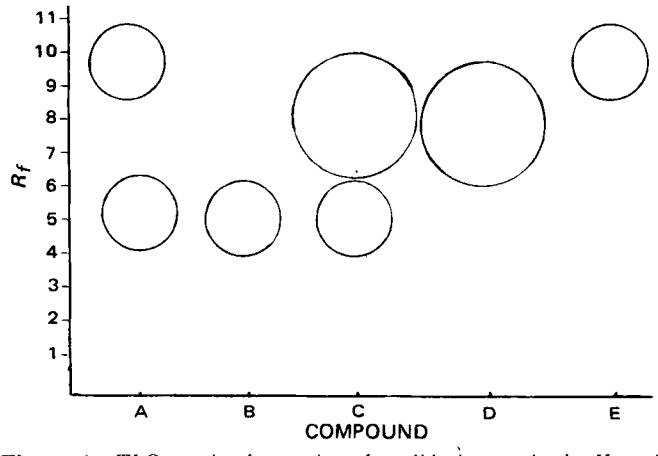

Figure 1—*TLC results for purity of antibiotic standards. Key: A, penicillin V potassium, proposed standard, 0.1 μg; B, penicillin V potassium, working standard, 0.1 μg; C, penicillin G potassium, 0.005 μg, and penicillin V potassium, 0.1 μg; D, penicillin G potassium, working standard, 0.005 μg; and E, p-hydroxypenicillin V potassium, 0.01 μg.*

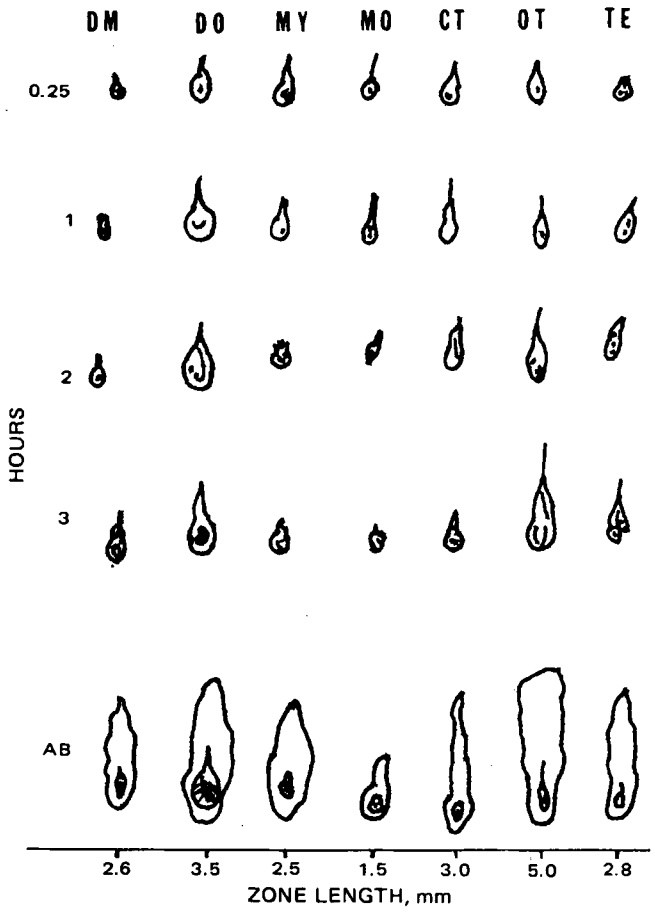

Figure 2—*Bioautography of seven antibiotic standards (left to right): DM, demeclocycline; DO, doxycycline; MY, methacycline; MO, minocycline; CT, chlortetracycline; OT, oxytetracycline; and TE, tetracycline. Top to bottom and left to right: 0.25 hr—yellow, pale yellow with pink tint, pale yellow, yellow, yellow, yellow, and yellow; 1 hr—yellow circle with purple beard, yellow center with pink outline, pale yellow, yellow, yellow, yellow, and yellow with pink beard; 2 hr—yellow center with purple beard, yellow center with rose outline, yellow center with pink outline, yellow, yellow center with gray beard, pale yellow, and orange center with purple beard; 3 hr—light brown, dark brown with yellow center, pale-orange outline with gray beard, bright yellow, bright yellow with tan beard, pale yellow, and gray. The spots obtained after the bioautograph are denoted by AB.*

The method described in the *Code of Federal Regulations* (paragraph 436.308) to identify the tetracyclines is a paper chromatographic technique in which individual tetracyclines are identified presumptively when the R_f value of the unknown matches the R_f value of a known tetracycline. During the development of this thin-layer technique, it was noted that, after the chromatographic procedure, a series of color changes takes place that is unique for each tetracycline spot. Each spot develops in an individual manner with regard to shape and color so that no two give the exact same pictures. After the bioautograph, no two zones have identical configurations. The combination of shape and color at the point of origin and individual shapes of the zones of inhibition can serve as a means of identification. As in the paper chromatographic method, an unknown sample can be run with the seven known standards and readily identified (Fig. 2).

Since this TLC system is quite adaptable and the biological phase is readily adjusted for particular needs, variations of the basic system should be applied success-

fully to other contamination problems and other areas of interest.

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BOOKS

REVIEWS

Drug Design, Vol. VIII. Edited by E. J. ARIENS. Academic, 111 Fifth Ave., New York, NY 10003. 1979. 420 pp. 15 × 23 cm. $42.00.

This book is the eighth member of a continuing set, which collectively comprises Volume 11 of the series of monographs entitled "Medicinal Chemistry." Dr. Ariens has produced another valuable book. As stated in the preface of this volume, the term "drug" is to be interpreted in the widest sense. Indeed, this volume includes such diverse agents as synthetic sweeteners, ionophores, and potential environmental pollutants.

In the first chapter, Martin discusses "Advances in the Methodology of Quantitative Drug Design." This chapter is a systematic survey of the approaches available for finding the substituent group giving optimum activity from a lead compound either by batch or stepwise selection of new derivatives. The parameters for describing electronic, steric, and, particularly, lipophilic effects of substituents are considered. Considerable attention is devoted to theoretical models for the distribution of drugs into different compartments depending on their physicochemical properties. Finally, the information that can be obtained by regression and discriminate analyses is discussed. The most valuable aspect of the chapter probably is the many caveats concerning the limitations and pitfalls of quantitative structure–activity relationship methods.

In the second chapter, Kirschner and Kowalski deal with the "Application of Pattern Recognition to Drug Design." The first portion covers the general methodology of pattern recognition, and the second portion covers applications to drugs. Although development of this powerful mathematical tool has been initiated, much more work is needed.

In Chapter 3, the design of drug delivery systems that will release a constant amount of drug over a long period is discussed by Chandrasekaran, Theeuwes, and Yum. This chapter primarily describes the features and theory behind three currently available systems; OROS Theophyllin for oral use, a transdermal scopolamine system, and the Alzet osmotic minipump. Chapter 4 is an excellent discussion of the use of receptor binding data for the design of steroid hormones by Raynaud, Ojasoo, Bouton, and Philibert. The general techniques of receptor isolation and displacement of bound, radiolabeled ligands for various hormonal activities are presented. Then, the specific structure–activity relationships for receptor binding of estrogens, progestins, androgens, and mineralcorticoid and glucocorticoid hormones are considered. The relationship between receptor binding and *in vivo* activity is discussed last.

The fifth chapter, authored by Crosby, DuBois, and Wingard, covers synthetic sweeteners. There is an interesting discussion of the theory of taste and the known structure–activity relationships for sweet substances; however, too much basic material on molecular interactions, Hansch treatment, *etc.*, is included that is covered elsewhere in the series and detracts from this chapter. Chapter 6, "Prospective Assessment of Environmental Effects of Chemicals," by Hueck-van der Plas and Hueck is concerned with the test systems that can be used to predict the environmental impact of chemicals. This chapter probably will be of more interest to researchers dealing with agricultural chemicals rather than to those concerned with drugs for human use.

The final chapter is fascinating; it describes the "Design of Selective Ion Binding Macrocyclic Compounds and Their Biological Applications" and was authored by Izatt, Lamb, Eatough, Christensen, and Rytting. The factors affecting the selective-ion complexation by crown ethers and derivatives are discussed in detail.

This volume generally is well written and free from typographical errors. Every medicinal chemist should find something of interest.

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Foreign Compound Metabolism in Mammals, Vol. 5. A Review of the Literature Published During 1976 and 1977. Senior Reporter, D. E. HATHWAY. The Chemical Society, Burlington House, London W1V OBN, England. 1979. 567 pp. 13 × 22 cm. Price $70.00. (Available from the American Chemical Society, 1155 16th St., N.W., Washington, DC 20036.)

This volume is an organized condensation of almost 3000 major metabolism–pharmacokinetics papers published in 1976 and 1977. The first half of the volume consists of five subject-oriented chapters, which are followed by eight product-oriented chapters.

Chapter 1, Drug Kinetics by P. G. Wellington, is the longest chapter (86 pages) and has the highest density of references (758 total references for a mean of 8.6 references/page). This chapter has 20 well-selected sections and deals with prostaglandins, ethanol, inorganic ions, and diagnostic agents in addition to a wide variety of drug classes. Chapter 2, Enzymic Mechanisms of Oxidation, Reduction, and Hydrolysis by P. Bentley and F. Oesch, interestingly covers the assigned terrain. Many readers will appreciate the subsection on epoxide hydratase for its inclusion of topics such as control and induction and occurrence in extrahepatic tissues.

P. C. Hirom and P. Millburn cover Enzymic Mechanisms of Conjugation (Chapter 3) in a fairly lively fashion. They briefly discuss new conjugation reactions and amino acid conjugations, and they animate their treatment of "the usual suspects" by reporting the tissue distribution of the enzymes responsible for conjugations with glucuronic acid, sulfate, and glutathione. In Chapter 4, J. D. Baty deals selectively and well with Species, Strain, and Sex Differences in Metabolism. Beside discussing comparative catabolic and conjugation reactions, Baty focuses on comparative differences in biliary excretion. The final subject-oriented chapter, Mechanisms of Chemical Carcinogenesis by D. E. Hathway, has this reviewer's enthusiastic endorsement for its interesting, valuable, and topical content. Its 54 pages utilize 358 well-chosen references that are integrated superbly.

The first product-oriented chapter has a more pharmacological orientation than do the others. This chapter, the Effect of Drugs on the Central Nervous System by B. E. Leonard, integrates mechanisms of action with biotransformations. C. Rhodes divides his report on Cardiovascular Drugs into sections dealing with thrombosis, hypertension, and cardiac disorders and summarizes some particularly interesting interspecies differences in biotransformation pathways of numerous drugs. L. G. Dring and P. Millburn address the metabolism of Sympathomimetic Amines and Bronchodilators and underscore many differences between *in vivo* and *in vitro* biotransformation routes.