At the concentration studied, alcohol and various glycols increase the viscosity of the gels to the same extent.

Compounds capable of interacting with CMC increase the viscosity of the gels.

Acetaminophen demonstrates extensive interaction with MCC-CMC.

REFERENCES

(1) Samyn, J. C., *J. Pharm. Sci.*, **50**, 517(1961).
(2) *Avicel RC Microcryst. Cellulose and Its Use in Pharm. Susp.*, Am. Viscose Div., FMC Corp., Marcus Hook, Pa., p. 1.
(3) Hermans, J., Jr., *J. Appl. Polymer Sci.*, **9**, 1973(1965).
(4) *Avicel RC Microcryst. Cellulose and Its Use in Pharm. Susp.*, Am. Viscose Div., FMC Corp., Marcus Hook, Pa., p. 2.
(5) Raynor, G. E., personal communication (July 6, 1966).
(6) Healy, T. W., and LaMer, V. K., *J. Colloid Sci.*, **19** 323(1964).
(7) Green, H., "Industrial Rheology and Rheological Structures," Wiley, New York, N. Y., 1949, p. 69.

Keyphrases

Microcrystalline cellulose (MCC)-carboxymethylcellulose (CMC)-gels
Rheology—MCC-CMC gels
Suspending adjuvants, effect—gels
Shear rate, stress range, effect—gels

Absorption, Metabolism, and Excretion of the Ephedrines in Man II

Pharmacokinetics

By G. R. WILKINSON* and A. H. BECKETT

The kinetics of absorption, metabolism, and excretion of (−)-norephedrine, (−)-ephedrine, and (−)-methylephedrine, after oral administration in aqueous solution, have been elucidated using analog computer analysis of urinary excretion data from three male subjects under constant acidic urine control. The kinetics of formation and elimination of norephedrine and ephedrine when present as metabolites have also been determined. The single body compartment mathematical models were based upon a catenary chain with parallel branch systems and all rate constants were assumed to be first order. Excellent agreement was obtained between the theoretical computer curves and the experimental excretion data for both unchanged drug and metabolite(s).

THE DEVELOPMENT of mathematical models to describe the absorption and fate of a drug and its metabolite(s) in the body, and the value of such models in the design of dosage forms and regimens has been treated by numerous authors including Teorell (1), Dominguez (2), Dost (3), Nelson (4), Wagner (5), Krüger-Thiemer (6) and references therein. For maximum exploitation of these techniques drug plasma levels are preferable, however, the distribution characteristics of many drugs, especially basic drugs (7), are such that this information is not readily available. In these cases it is possible to use urinary excretion data providing caution is taken in the interpretation of the results (8, 9).

In view of the long and extensive use of the ephedrines[1] it is surprising that relatively little is known about the kinetics of their absorption and elimination in man. The only published information concerns (−)-norephedrine, where the mean elimination half-life was reported to be 3.9 hr. (10). Recent publications (11, 12) have indicated that the relative metabolism and urinary excretion of the ephedrines are dependent upon the urinary pH and in certain circumstances the volume also. The validity of urinary excretion kinetics is based upon the assumption that the excretion rate reflects the plasma concentration of the drug. With drugs exhibiting non-

Received March 15, 1968, from the Department of Pharmacy, Chelsea College of Science and Technology, University of London, London, S.W. 3, England.
Accepted for publication August 9, 1968.
Based on thesis submitted by G. R. Wilkinson to the University of London in partial fulfillment of Doctor of Philosophy degree requirements.
* Present address: College of Pharmacy, University of Kentucky, Lexington, KY 40506

[1] The term ephedrines will be used to describe collectively the levo isomers of norephedrine, ephedrine, and methylephedrine.

ionic reabsorption in the kidney tubule, this requirement will only be fulfilled when reabsorption of the drug back into the body is either absent or negligible. Such a condition exists for the ephedrines when the urine is acidic at about pH 5.0 (12).

This communication reports the kinetics of absorption, metabolism, and excretion of (−)-norephedrine, (−)-ephedrine, and (−)-methylephedrine after oral administration in aqueous solution. When norephedrine and ephedrine were present as metabolites the kinetics of their formation and elimination were also elucidated. The kinetic parameters were obtained by analog computer analysis of urinary excretion data obtained in a previous study, where the urinary pH was maintained acidic by the administration of ammonium chloride (12).

EXPERIMENTAL

The data for analysis consisted of 24-hr. cumulative urinary excretion and rate of excretion values for unchanged norephedrine, ephedrine, and methylephedrine after their oral administration to three healthy male volunteers. In the case of ephedrine and methylephedrine, metabolite excretion data were also available, *i.e.*, norephedrine and ephedrine and norephedrine formed from ephedrine and methylephedrine, respectively (12). These raw data allowed the development of simple models to describe the absorption, metabolism, and excretion of the three drugs when administered *per se*. Where norephedrine and ephedrine were present in the body as a consequence of metabolism, it was assumed that their fate was identical to that postulated after *per se* oral administration. The three models are shown in Fig. 1 while the assumptions and terminology used in their development are given in the *Appendix*.

The analog computer used was a Pace TR 20R (Electronics Associates Ltd.) along with an X-Y recorder (Advance Electronics Ltd.) and a digital voltmeter (Roband Ltd.). Standard methods were used to program the particular model under investigation and also to fit the generated curves to the experimental data (13). Both the cumulative and rate curves for excretion were used to obtain the best "goodness of fit." As a further aid to fitting, the absorption curve derived from the data by the method of Wagner and Nelson (14) was also used.

Lag time was estimated by extrapolating the derived curve for the amount of drug remaining to be absorbed, plotted semilogarithmically, to zero absorption, assuming absorption to be a single first-order process. This lag time was not programmed into the computer, instead the abscissa zero of the X-Y recorder was manually set at the lag time prior to any simulation.

In fitting the norephedrine excretion curves after the administration of (−)-ephedrine, it was assumed that although the value for the overall elimination constant *ky* might be different from that determined after *per se* administration, the ratio of the component rate constants, *kp* and km_3, would remain constant for each particular subject. A similar assumption was made for the overall elimination constant *kz* when fitting the ephedrine excretion curves after administration of (−)-methylephedrine. For subjects N.B. and G.R.W. the results for *ku*/*kz*, km_3/kz, and km_4/kz derived from the data obtained after administration of 20.48 and 25.00 mg. (−)-ephedrine were averaged and these values used in the (−)-methylephedrine simulation. For subject R.W.D., only the results obtained from the 20.48-mg. dose were used. In all cases the metab-

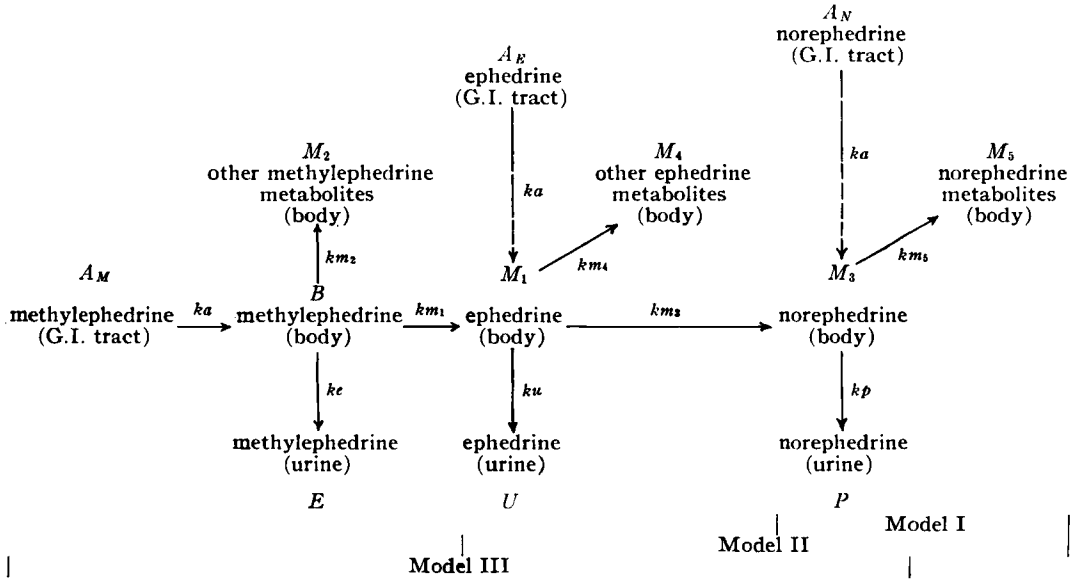

Fig 1—Pharmacokinetic models for the absorption, metabolism, and excretion of (−)-norephedrine (Model I), (−)-ephedrine (Model II), and (−)-methylephedrine (Model III) in man.

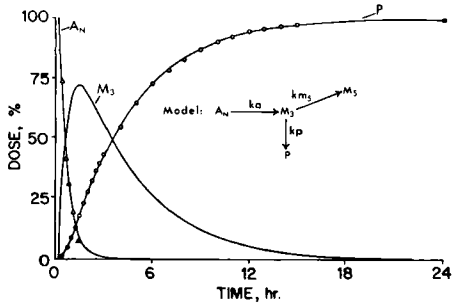

Fig. 2—Computer curves and experimental data points for the elimination, after oral administration, of (−)-norephedrine (subject, N.B.).

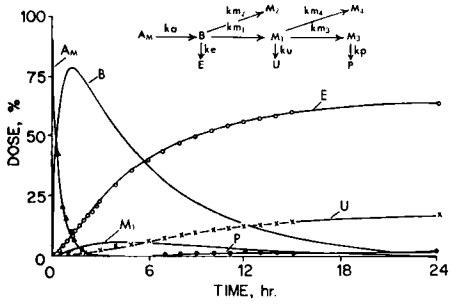

Fig. 4—Computer curves and experimental data points for the elimination, after oral administration, of (−)-methylephedrine (subject, N.B.).

olism of the norephedrine, formed after (−)-methylephedrine administration, was neglected and it was assumed that all of the drug was excreted unchanged in the urine, *i.e.*, *kp* becomes *ky*.

RESULTS AND DISCUSSION

Excellent agreement, for both unchanged drug and metabolite(s), was obtained between the fitted computer urinary excretion curves and the experimental data for all three amines, indicating the suitability of the postulated models to describe, within experimental error, the kinetics of absorption, metabolism, and excretion of these drugs. Typical fitted cumulative excretion curves along with various derived curves for some other compartments are shown in Figs. 2–4. The kinetic parameters for each subject are summarized in Tables I, II, and III.

Absorption of all three amines after oral administration could be described, with one exception, by a single first-order process following a lag period. Considerable intersubject and, in the case of (−)-ephedrine, intrasubject variation was observed in both the lag time and the rate constant for absorption. No correlation was apparent between these variations and either the drugs or the subjects. However, despite these variations all the ephedrines were rapidly absorbed within 3 hr. of administration. The absorption of (−)-norephedrine in subject G.R.W. appeared to be biphasic, a slow phase followed by a much faster phase, rather than mono-exponential. The computer curve was fitted only to the latter process accounting for the long lag time (67 min.). A number of physiological explanations may be proposed to describe these absorption profiles, however, until more detailed information than that presented herein is available, these would be purely speculative and of questionable significance.

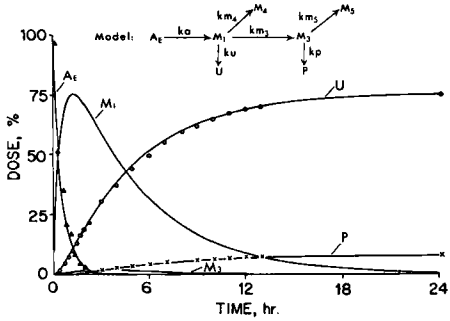

Fig. 3—Computer curves and experimental data points for the elimination; after oral administration, of (−)-ephedrine (subject, R.W.D.).

In the postabsorption phase, the elimination of unchanged drug, whether norephedrine, ephedrine, or methylephedrine, could be described by a series of simultaneous, competitive first-order processes—excretion and metabolism to one or more metabolite(s). As might be expected, intersubject and intrasubject variations occurred in the values of the rate constants for these processes. However, in the case of ephedrine, the ratio of the constants was remarkably constant within subjects N.B. and G.R.W. In all cases, the rate constants for urinary excretion of unchanged norephedrine (*kp*) and ephedrine (*ku*) were of the same magnitude, but the equivalent constant for methylephedrine (*ke*) was always significantly smaller. This was also apparent in the rate constants for overall elimination of the drug. Differences in the volume of distribution and/or urinary excretion behavior of methylephedrine compared to norephedrine and ephedrine may account for this finding. Despite these differences, under the experimental conditions used in the study, all three of the ephedrines and their measured metabolite(s) were rapidly eliminated from the body. For example, mean overall elimination $t_{1/2}$ values for unchanged drug were: (−)-norephedrine, 2.99 hr.; (−)-ephedrine, 3.03 hr.; and (−)-methylephedrine, 4.49 hr.

As stated above, the formation of the metabolites, ephedrine and norephedrine, after the administration of both (−)-ephedrine and (−)-methylephedrine, was described by first-order processes. The phar-

TABLE I—KINETIC PARAMETERS FOR THE ABSORPTION, METABOLISM, AND EXCRETION OF (−)-NOREPHEDRINE

Subject	Lag Time, min.	*ka*, hr.$^{-1}$	*kp*, hr.$^{-1}$	*km*$_5$, hr.$^{-1}$	*ky*, hr.$^{-1}$
N.B.	15	1.80	0.2581	0.0008	0.2589 (2.67)[a]
G.R.W.	67	2.00	0.2222	0.0210	0.2432 (2.85)
R.W.D.	8	9.24	0.1951	0.0063	0.2014 (3.44)

[a] Values in parentheses are the $t_{1/2}$ values, in hr., equivalent to the rate constant above.

TABLE II—KINETIC PARAMETERS FOR THE ABSORPTION, METABOLISM, AND EXCRETION OF (−)-EPHEDRINE

Subject	Dose Ephedrine, mg.	Lag Time, min.	ka, hr.$^{-1}$	ku, hr.$^{-1}$	km_2, hr.$^{-1}$	km_4, hr.$^{-1}$	kz, hr.$^{-1}$	kp, hr.$^{-1}$	km_3, hr.$^{-1}$	ky, hr.$^{-1}$
N.B.	20.48	15	1.75	0.2180	0.0220	0.0099	0.2499 (2.77)[a]	0.3588	0.0012	0.3600 (1.92)
	25.00	18	4.02	0.2172	0.0256	0.0096	0.2524 (2.74)	0.3400	0.0010	0.3410 (2.03)
G.R.W.	20.48	12	3.30	0.2566	0.0124	0.0060	0.2750 (2.52)	0.3290	0.0310	0.3600 (1.92)
	25.00	15	2.77	0.1780	0.0074	0.0056	0.1910 (3.63)	0.2741	0.0259	0.3000 (2.31)
R.W.D.	20.48	0	3.00	0.1933	0.0180	0.0012	0.2125 (3.26)	0.4068	0.0132	0.4200 (1.65)
	25.00	6	1.85	0.1596	0.0174	0.0337	0.2107 (3.29)	0.4940	0.0160	0.5100 (1.36)

[a] Values in parentheses are the $t_{1/2}$ values, in hr., equivalent to the rate constant above.

macokinetic models found to represent the elimination of these drugs after oral administration *per se* were also found suitable when the amines were present as metabolites. However, initial attempts to fit the theoretical urinary excretion curves of the metabolites using the same values for the rate constants as obtained from the *per se* administration data were unsuccessful. In all cases the rate of excretion was too small, even if urinary excretion was assumed to be the only elimination pathway. The only way of fitting the data was to increase, often substantially, each rate constant contributing to the overall elimination, assuming that the constants remained in the same ratio as after *per se* administration (the results from the two separate ephedrine trials would support the hypothesis of a constant ratio despite changes in the absolute values of the constants). For example, to fit the norephedrine excretion data after (−)-ephedrine administration the individual rate constants for elimination, ky, had to be increased as follows: N.B., 32 and 39%; G.R.W., 23 and 48%; and R.W.D., 109 and 153%. Similar increases for ephedrine (kz), based upon the mean value for the two *per se* trials, and norephedrine (ky) after (−)-methylephedrine administration were respectively: N.B., 12 and 34%; G.R.W., 33% (norephedrine not determined); and R.W.D., 155 and 60%.

Insufficient data prevent any statistical significance being placed upon these results, however, the authors consider increases of over 150% are well beyond the usual intrasubject variations found in individuals, particularly when an acidic urine is being maintained by ammonium chloride. This being the case, then it would appear that ephedrine and norephedrine, when present in the body as metabolites, are eliminated faster than when they are administered *per se*.

A possible explanation for this phenomenon is that the kinetics of elimination of the ephedrines are dose dependent; elimination is faster at low body drug levels than at high levels. Such a change should be apparent in the logarithmic excretion curve of unchanged drug if sampling is continued beyond the time that the critical drug level occurs. However, examination of the *per se* experimental data for norephedrine and ephedrine, over a time period greater than five orders of magnitude of the elimination half-life, does not show any deviation from a mono-exponential curve (12). In the present study, the values for the excretion rate of unchanged drug, whether norephedrine or ephedrine, did not completely extend into the range of values obtained when the drugs were excreted as metabolites; the 12–16 hr. values for unchanged drugs were approximately equal to the maximal rates found when the drug was a metabolite. Hence, it is possible that the critical body level of drug, after *per se* administration, had not been reached before hourly urine collection was terminated.

In the development of the present pharmacokinetic models it was necessary to assume that the body behaved as a single homogeneous compartment. Such a gross oversimplification may provide an alternative explanation for the elimination behavior of norephedrine and ephedrine when present in the body as metabolites. The shortcomings of conceiving the body to exhibit properties of a single compartment have recently been reemphasized by Riegelman *et al.* (15). These authors suggest that for many drugs it is more reasonable to consider the body as composed of a cen-

TABLE III—KINETIC PARAMETERS FOR THE ABSORPTION, METABOLISM, AND EXCRETION OF (−)-METHYLEPHEDRINE

Subject	Lag Time, min.	ka, hr.$^{-1}$	ke, hr.$^{-1}$	km_1, hr.$^{-1}$	km_2, hr.$^{-1}$	kd, hr.$^{-1}$	ku, hr.$^{-1}$	km_3, hr.$^{-1}$	km_4, hr.$^{-1}$	kz, hr.$^{-1}$	kp, hr.$^{-1}$
N.B.	6	2.00	0.1145	0.0345	0.0260	0.1750 (3.96)[a]	0.2426	0.0274	0.0100	0.2800 (2.47)	0.3462 (2.00)
G.R.W.	36	2.60	0.1112	0.0166	0.0122	0.1400 (4.95)	0.2862	0.0150	0.0088	0.3100 (2.23)	—
R.W.D.	15	4.60	0.1283	0.0237	0.0000	0.1520 (4.56)	0.4912	0.0457	0.0031	0.5400 (1.28)	0.2820 (2.45)

[a] Values in parentheses are the $t_{1/2}$ values, in hr., equivalent to the rate constant above.

tral compartment and at least one peripheral compartment. That such a system may be applied to the ephedrines is suggested by the data obtained after intravenous administration (12) and also by the fact that in some cases after oral administration a distribution nose is observed in the rate of excretion curve of unchanged drug.

In a two-compartmental open system, the identification of a particular exponential term with a particular process is erroneous, as all observed rate constants are hybrid constants each influenced by the factors controlling drug distribution and elimination. Hence, all of the rate constants determined in this study are apparent constants influenced not only by the intrinsic rate constants but also by the volumes of the central and peripheral compartments, and therefore the rate constants for distribution between these compartments. A change in the value of any of these factors will affect the observed rate constants. Such changes probably explain the observed intrasubject variations in the calculated constants and possibly the increased values obtained when a drug is present as a metabolite. This latter phenomenon and the mechanism by which the elimination of a metabolic ephedrine is influenced by the presence of a parent ephedrine is presently under further investigation.

Even within the small group of subjects used in this study, large inter- and intrasubject variations occurred in the various rate constants. However, it is considered that by using a small but well controlled number of subjects more meaningful and valuable information can be obtained compared to the use of larger groups, which although statistically more satisfying do not control the urinary pH.

It would be expected that the overall elimination of unchanged ephedrine would be faster when the urinary pH was maintained acidic than when no control was attempted, and this probably explains the difference between the present data for (−)-norephedrine and that of Heimlich *et al.* (10). However, the differences between the data are not large on account of the low pH-sensitivity of (−)-norephedrine (12, 16).

A kinetic study such as that presented in this paper is an oversimplification of complex physiological phenomena. Furthermore, kinetic parameters obtained under extreme urinary pH conditions are not applicable to normal conditions, especially if drug absorption, distribution, binding, *etc.*, are affected by acidification of the urine. However, if the limitations of the techniques are recognized, then they may be used advantageously for comparative drug metabolism studies and the determination of absorption profiles from both conventional and prolonged-release dosage forms, particularly for drugs which are extensively extravascularly distributed (17).

APPENDIX

Assumptions

1. Transfer from one compartment to another is irreversible, the rate constants for transfer being first order with the units of reciprocal time.

2. The compartments are uniform and homogeneous during the processes and equilibration is instantaneous.

3. There is no decomposition of drug at the absorption site, no enterohepatic recycling, no diffusion of drug from the blood into the stomach.

4. Drug is immediately and completely available at the absorption site at zero time and is 100% absorbed (12).

Definitions

Lag time: time interval between ingestion of the drug and zero time.

Zero time: time at which drug appearing in the body may be described by a single first-order process.

A_M, A_E, A_N: amount of methylephedrine, ephedrine, or norephedrine, respectively, present in the gastrointestinal tract.

B, M_1, M_3: amount of methylephedrine, ephedrine, and norephedrine, respectively, present in the body.

M_2: amount of metabolite(s) of methylephedrine other than ephedrine and/or methylephedrine eliminated by a pathway other than the urine.

M_4: amount of metabolite(s) of ephedrine other than norephedrine and/or ephedrine eliminated by a pathway other than the urine.

M_5: amount of metabolite(s) of norephedrine and/or norephedrine eliminated by a pathway other than the urine.

E, U, P: amount of methylephedrine, ephedrine, and norephedrine, respectively, in the urine.

ka: rate constant for the appearance of drug from the gastrointestinal tract into the body whether methylephedrine, ephedrine, or norephedrine.

ke, ku, kp: rate constant for excretion of methylephedrine, ephedrine, and norephedrine, respectively, from the body into the urine.

km_1: rate constant for the formation of ephedrine from methylephedrine.

km_2: rate constant for the formation of metabolite(s) of methylephedrine other than ephedrine and/or rate constant for elimination of methylephedrine by a pathway other than the urine.

km_3: rate constant for the formation of norephedrine from ephedrine.

km_4: rate constant for the formation of metabolite(s) of ephedrine other than norephedrine and/or rate constant for elimination of ephedrine by a pathway other than the urine.

km_5: rate constant for the formation of metabolite(s) of norephedrine and/or rate constant for elimination of norephedrine by a pathway other than the urine.

kd, kz, ky: rate constant for the overall elimination from the body by all pathways of methylephedrine, ephedrine, and norephedrine, respectively, *i.e.*, $kd = ke + km_1 + km_2$; $kz = ku + km_3 + km_4$; $ky = kp + km_5$.

REFERENCES

(1) Teorell, T., *Arch. Intern. Pharmacodyn.*, **57**, 205 (1937).
(2) Dominguez, R., "Kinetics of Elimination, Absorption and Volume of Distribution in the Organism," Medical Physics II, Yearbook Publishers, Inc., Chicago, Ill., 1950, pp. 476–489.
(3) Dost, F. H., "Der Blutspiegel," Thieme, Leipzig, 1953.
(4) Nelson, E., *J. Pharm. Sci.*, **50**, 181(1961).
(5) Wagner, J. G., *ibid.*, **50**, 359(1961).
(6) Krüger-Thiemer, E., *J. Theoret. Biol.*, **13**, 212(1966).
(7) Way, E. L., and Adler, T. K., *Bull. World Health Organ.*, **27**, 359(1962).
(8) Wagner, J. G., *J. Pharm. Sci.*, **52**, 1097(1963).
(9) Martin, B. K., *Brit. J. Pharmacol.*, **29**, 181(1967).
(10) Heimlich, K. R., MacDonnell, D. R., Flanagan, T. L., and O'Brien, P. D., *J. Pharm. Sci.*, **50**, 232(1961).
(11) Beckett, A. H., and Wilkinson, G. R., *J. Pharm. Pharmacol.*, **17**, 107S(1965).
(12) Wilkinson, G. R., and Beckett, A. H., *J. Pharmacol. Exptl. Therap.*, **162**, 139(1968).
(13) Jackson, A. S., "Analog Computation," McGraw-Hill, New York, N. Y., 1960.
(14) Wagner, J. G., and Nelson, E., *J. Pharm. Sci.*, **52**, 610(1963).
(15) Riegelman, S., Loo, J., and Rowland, M., *ibid.*, **57**, 117(1968).
(16) Wilkinson, G. R., and Beckett, A. H., in press.
(17) Beckett, A. H., and Tucker, G. T., *J. Pharm. Pharmacol.*, **18**, 72S(1966).

Keyphrases

Ephedrines—pharmacokinetics
Pharmacokinetics—ephedrines, absorption, metabolism, excretion
Models, single compartment—ephedrines, pharmacokinetics
Metabolites, ephedrines—formation, elimination

Stilbene Isothiocyanates as Potential Fluorescent Tagging Agents

By J. E. SINSHEIMER, J. T. STEWART*, and J. H. BURCKHALTER

Two groups of stilbene isothiocyanate derivatives, α-phenylcinnamic acids and α-phenylcinnamonitriles, have been synthesized. These compounds and their required intermediates were studied as to their structure-fluorescence relationships in an effort to develop a protein-tagging agent with blue fluorescence.

THE FLUORESCENT labeling of proteins as first developed by Coons *et al.* (1) and advanced by the introduction of fluorescent isothiocyanates by Burckhalter and co-workers (2, 3) has had extensive application to biological problems especially for the identification of pathogenic organisms. The agents and techniques employed together with their applications have been reviewed by Nairn (4). In varying degrees, all the presently available reagents can be improved upon in regard to purity, expense, fluorescent intensity, and stability to UV irradiation. It would also be highly desirable to have agents with contrasting fluorescent colors. For example, a blue fluorescent label would be valuable to supplement the green of fluorescein and orange to red fluorescence of rhodamine derivatives.

Limited studies with blue fluorescent tagging agents have employed stilbene optical brightening agents and a coumarin isocyanate (5) as well as β-anthryl isocyanate (6) with limited success.

Peck and Creech (7) synthesized 4′-isocyanato-4-dimethylaminostilbene for the purpose of combining it with proteins for UV analysis, but no mention was made of the possibility of utilizing the blue fluorescent properties of the stilbene and resulting protein conjugate. A blue fluorescent reagent, the disodium salt of 4′-acetamido-4-isothiocyanatostilbene-2,2′-disulfonic acid, has been described for the specific labeling of the outer components of the plasma membrane, but it has not been applied to antibody labeling (8).

Successful use of stilbene compounds as blue fluorescent optical brightening agents (9) of high stability encouraged the authors to seek improved fluorescent labeling agents among stilbene derivatives. The purpose of this investigation was to synthesize a stilbene isothiocyanate suitable for protein labeling as well as to study structure-fluorescence relationships in a series of model stilbene compounds. More specifically a stilbene isothiocyanate protein-tagging agent was postu-

Received June 10, 1968, from the College of Pharmacy, University of Michigan, Ann Arbor, MI 48104
Accepted for publication August 8, 1968.
Abstracted in part from a dissertation submitted by J. T. Stewart in partial fulfillment of the Doctor of Philosophy degree requirements.
This investigation was supported in part by research grant AI 05817 and training grant GM 1367, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
* Fellow of the American Foundation for Pharmaceutical Education: holder of the 1965–67 H. A. B. Dunning Memorial Fellowship. Present address: School of Pharmacy, University of Georgia, Athens, Ga.