When a sample of vitamin D_3 was exposed to tritium gas at room temperature, no useful product was isolated, indicating that the use of low temperature (−198°) for labeling by exposure to tritium gas is of marked advantage since the formation of radioactive impurities is decreased. Because β labeling is due to formation of excited and ionized molecular species (18) which require energy of activation for subsequent chemical reactions, it is reasonable to postulate that at −198°, the small RT value in the Boltzmann factor reduces the probability of reaction. In addition, recombination of molecular ions is facilitated because their diffusion in solids is restricted as a result of an enhancement of the reaction cage effect at −198°.

The validity of the latter factor is supported by the work of Wenzel, *et al.* (19), who noted an increase in specific activity as well as fewer radioactive impurities after gas exposure labeling of charcoal-adsorbed compounds compared to the nonadsorbed control compounds. It is probable that either charcoal adsorption or low temperature (−198°) brings about a common effect, *i.e.*, a passive rigidity of molecular configuration and a restricted diffusion of molecular ions; both of these effects tend to diminish radiation decomposition and to increase substitution by tritium during exposure.

REFERENCES

(1) Wilzbach, K. E., *J. Am. Chem. Soc.*, **76**, 1448(1957).
(2) Jones, E. P., Mason, L. H., Dutton, H. J., and Nystrom, R. F., *J. Org. Chem.*, **25**, 1413(1960).
(3) Dutton, H. J., and Nystrom, R. F., *Proc. Symp. Tritium Tracer Appl.*, **1958**, 8.
(4) Whisman, M. L., *Anal. Chem.*, **33**, 1284(1961).
(5) Dutton, H. J., Jones, E. P., Davison, V. L., and Nystrom, R. F., *J. Org. Chem.*, **27**, 2648(1962).
(6) Pratt, T. H., and Wolfgang, R., *J. Am. Chem. Soc.*, **83**, 10(1961).
(7) Yang, K., and Gant, P. L., *J. Chem. Phys.*, **31**, 1589 (1959).
(8) *Ibid.*, **66**, 1619(1962).
(9) Wilzbach, K. E., in "Tritium in the Physical and Biological Sciences," International Atomic Energy Agency, Vienna, Austria, 1962, p. 3.
(10) Stahl, E., *Chem. Zeit.*, **82**, 323(1958).
(11) Grodsky, G. M., Carbone, J. V., Fanska, R., and Peng, C. T., *Am. J. Physiol.*, **203**, 532(1962).
(12) Mulder, F. J., Roborgh, J. R., deMan, Th. J., Keuning, K. J., and Hanewald, K. H., *Rec. Trav. Chim.*, **76**, 733 (1957).
(13) McMahon, J. M., Davis, R. B., and Kalinitsky, G., *Proc. Soc. Exptl. Biol. Med.*, **75**, 799(1950).
(14) Peng, C. T., *Anal. Chem.*, **32**, 1292(1960).
(15) Dorfman, L. M., *Phys. Rev.*, **95**, 393(1954).
(16) Slack, L., and Way, K., "Radiation from Radioactive Atoms," U. S. Atomic Energy Commission, Washington 25, D. C., 1959, p. 3.
(17) Smyth, F. S., Reilly, W. A., Scott, K. G., Peng, C. T., and Stevenson, E., Abstracts of the Tenth Annual Meeting of the Western Society for Pediatric Research, Olympic Hotel, Seattle, Wash., November 1962, paper 45.
(18) Ache, H. J., Herr, W., and Thiemann, A., in "Tritium in the Physical and Biological Sciences," International Atomic Energy Agency, Vienna, Austria, 1962, p. 27.
(19) Wenzel, M., Wollenberg, H., and Schulze, P. E., *ibid.*, p. 37.

Kinetics of the Metabolism of Acetaminophen by Humans

By EINO NELSON and TADASHI MORIOKA

The kinetics of the metabolism of acetaminophen to its sulfuric and glucuronic acid conjugates has been studied in normal adult humans. In post-absorptive and post-equilibrative times the elimination process was found to be first order with a mean half-life of 1.95 hours with a range of 1.62 to 2.83 hours in nine tests using a five-subject test panel. Previously published data on pain threshold elevation resulting from ingestion of acetaminophen were examined during times following maximum elevation. Decay of pain threshold elevation was shown to be an apparent first-order process with a half-life of 0.42 hours. This half-life was less than one-fourth the half-life for metabolism, indicating that poor correlation exists between pharmacological activity and body level of acetaminophen, even though a kinetic relationship may exist between these quantities.

THE ANALGESIC DRUG, acetaminophen (4′-hydroxyacetanilide), is known to be metabolized nearly completely after administration to humans. Information on the metabolic fate of this drug in humans has been summarized by Williams (1). About 3% of an oral dose is excreted unchanged in the urine, and most of the balance of a dose can be found in the urine conjugated with sulfuric and glucuronic acids, with the latter conjugate predominating. The conjugates are formed at the 4′-hydroxy position on the molecule. Apparently the kinetics of the metabolism of this substance have not been studied previously. This communication reports the results of a pharmacokinetic study of the metabolism and excretion of acetaminophen and discusses the results obtained in terms of the physiological and pharmacological factors involved.

Received December 19, 1962, from the Pharmacokinetics Laboratory, School of Pharmacy, State University of New York, Buffalo, and School of Pharmacy, University of California Medical Center, San Francisco.
Accepted for publication February 8, 1963.
Support in part by a grant-in-aid from the Lilly Research Laboratories, Indianapolis, Ind., is gratefully acknowledged.

EXPERIMENTAL

Subject and Test Procedure.—One-gram doses of drug grade acetaminophen (finely powdered by

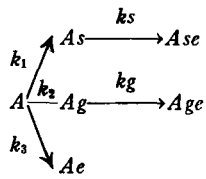
grinding in a mortar with a pestle and filled in hard gelatin capsules) were ingested in the morning on overnight-fasted stomachs by adult humans apparently in normal health. No food was taken until at least 1 hour after ingestion of the drug. The designation, sex, age, weight in kilograms, and body surface in sq. m. of the test subjects, respectively, were E-M-43-77-2.50, V-M-22-68-1.84, T-M-29-60-1.60, G-M-28-59-1.70, and S-F-37-57-1.62. Body surface areas were obtained from a nonogram (2) relating this quantity to height and weight. For determining blank excretion, urine specimens over a timed interval were obtained immediately prior to ingestion of the doses. Following ingestion of the doses, collection of urine at appropriate times was quantitative during the active course of the experiments. Nine experiments were made using the five-subject test panel.

Analytical Method.—The urines were assayed in triplicate for free acetaminophen by the method of Brodie and Axelrod (3) and in triplicate for total acetaminophen by a modification of the method described in the same publication. For determination of total acetaminophen, hydrolysis was effected by making suitably diluted urine 6 *N* in titer with hydrochloric acid and heating for 1.5 hours at 100°. One complete set of urines was assayed by both the modified and original method. Cumulative excretion of total drug was 942 mg. by the original method and 975 mg. by the modified method. The precision of the modified method was of the same order as the original method. The acetaminophen used to construct the standard curves was from the same lot used in the *in vivo* experiments.

RESULTS AND DISCUSSION

Cumulative free and conjugated drug excretions are listed in Table I.

Excretion Kinetics.—It was assumed that Scheme I described the kinetics of the metabolism and excretion of acetaminophen and its metabolites after absorption of a dose and attainment of apparent equilibrium between drug in blood and drug in other fluids of distribution



Scheme I

In Scheme I, A is the amount of acetaminophen in the body at any time; As and Ag are the amounts of acetaminophen in the form of its sulfuric and glucuronic acid conjugates, respectively, in the body at any time; Ase and Age are the amounts of acetaminophen sulfate and glucuronide ethers excreted in the urine in any time; and Ae is the amount of unchanged acetaminophen excreted in any time. The k's with number or letter subscripts are first-order rate constants with the dimensions of reciprocal time for the processes indicated. First-order processes for metabolism and urinary excretion are assumed since it has been found that this type of process describes within experimental error (in post-absorptive and post-equilibrative times) the metabolism and disposition of many drugs and foreign substances (4–15).

A preliminary examination of urinary excretion data indicated that apparent first-order excretion was being followed 2 or 3 hours after ingestion of doses. For mathematical treatment of data, a zero time shift (13, 14) of these values of time was made and cumulative amounts of drug excreted corrected to agree with the new time base by subtraction of the amount excreted in the time equal to the time shift.

TABLE I.—CUMULATIVE AMOUNTS OF FREE (F) AND CONJUGATED (C) ACETAMINOPHEN EXCRETED AT VARIOUS TIMES[a]

	1 Hr.		2 Hr.		3 Hr.		4 Hr.		5 Hr.		6 Hr.		7 Hr.		8 Hr.		9 Hr.		Terminal[b]	
Subject	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C
E-1	1	16	3	126	8	321	11	529	13	628	15	714	16	792	17	822	17	841	21	900
E-2	6	40	9	160	15	380	21	568	24	677	26	785	27	847	..	...	28	903	28	942
E-3	7	46	11	170	18	396	25	580	28	692	31	802	33	865	..	...	35	922	36	965
E-4	..	...	21	293	24	396	30	616	34	717	38	822	40	873	..	...	42	935	44	995
E-5	8	29	20	173	28	334	33	479	36	577	38	666	42	726	43	761	45	799	48	915
V	2	41	11	285	22	492	30	665	36	805	44	926	45	967	47	1010	49	1040	51	1081
G	0	100	6	287	11	490	13	645	15	747	17	827	18	865	19	900	20	933	21	969
T	5	0	9	169	13	332	16	463	19	558	22	628	..	...	24	710	..	...	35	904
S	..	...	13	205	..	...	24	586	26	716	27	806	..	...	..	...	28	911	29	942

[a] All data expressed as mg. of free drug. [b] Between 12 and 24 hours depending on a given subjects' sampling schedule.

TABLE II.—RATE CONSTANTS AND HALF-LIVES FOUND FOR THE METABOLISM AND EXCRETION OF ACETAMINOPHEN BY HUMANS[a]

Test Subjects	E-1	E-2	E-3	E-4	E-5
Rate constant (K), $hr.^{-1}$	0.400 (2.69×10^{-2})	0.385 (3.77×10^{-2})	0.394 (3.83×10^{-2})	0.427 (4.36×10^{-2})	0.275 (8.37×10^{-3})
Half-life, hr.	1.73 (1.14×10^{-1})	1.80 (1.79×10^{-1})	1.76 (1.85×10^{-1})	1.62 (1.60×10^{-1})	2.52 (3.60×10^{-2})
Test Subjects	**V**	**G**	**T**	**S**	**Mean**
Rate constant (K), $hr.^{-1}$	0.385 (4.58×10^{-2})	0.391 (1.90×10^{-2})	0.245 (1.28×10^{-2})	0.408 (3.73×10^{-2})	0.368 (5.60×10^{-2})
Half-life, hr.	1.85 (2.44×10^{-1})	1.77 (8.37×10^{-2})	2.83 (1.61×10^{-1})	1.70 (1.57×10^{-1})	1.95 (2.26×10^{-1})

[a] Standard deviations shown in parentheses.

In the model above it was further assumed that excretion of the metabolites of acetaminophen would be rate limited by the formation steps ($k_e \gg k_1$ and $k_g \gg k_2$).

On the basis of the considerations discussed in the preceding paragraphs, the following differential equations describe the several processes shown in the model

$$\frac{dA}{dE} = -KA \qquad \text{(Eq. 1)}$$

$$\frac{dAse}{dt} = k_1A \qquad \text{(Eq. 2)}$$

$$\frac{dAge}{dt} = k_2A \qquad \text{(Eq. 3)}$$

$$\frac{dAe}{dt} = k_3A \qquad \text{(Eq. 4)}$$

In Eq. 1 $K = k_1 + k_2 + k_3$. Equation 1 when integrated with constant of integration evaluated at zero time, where A° is the amount of acetaminophen in the body at zero time gives

$$A = A^\circ \, exp\,[-Kt] \qquad \text{(Eq. 5)}$$

Substitution in Eq. 2 of the value for A given in Eq. 5, and integration of the resulting expression with constant of integration evaluated at zero time under the condition that no metabolite was excreted gives

$$Ase = \frac{k_1A^\circ}{K}(1 - exp\,[-Kt]) \qquad \text{(Eq. 6)}$$

By a procedure identical with the immediately preceding one, Eq. 3 becomes

$$Age = \frac{k_2A^\circ}{K}(1 - exp\,[-Kt]) \qquad \text{(Eq. 7)}$$

and Eq. 4 becomes

$$Ae = \frac{k_3A^\circ}{K}(1 - exp\,[-Kt]) \qquad \text{(Eq. 8)}$$

In the experimental procedure adopted for following excretion of sulfuric and glucuronic acid ethers of acetaminophen, both substances were determined and the relative amounts of each form were unknown. Therefore, the combined excretion should be given by the sum of Eqs. 6 and 7

$$Am = \frac{k_1A^\circ}{K}(1 - exp\,[-Kt]) + \frac{k_2A^\circ}{K}(1 - exp\,[-Kt]) \qquad \text{(Eq. 9)}$$

In Eq. 9 Am is the amount of the sulfuric and glucuronic acid conjugates of acetaminophen excreted in the urine at any time. After a sufficiently long time corresponding to the time that only negligible excretion of metabolites occurs, where Am° is total amount of metabolites excreted after zero time, Eq. 9 reduces to

$$Am^\circ = \frac{k_1A^\circ}{K} + \frac{k_2A^\circ}{K} \qquad \text{(Eq. 10)}$$

If the value of the constants on the right-hand side of Eq. 10 are substituted in Eq. 9, the latter equation becomes

$$Am = Am^\circ(1 - exp\,[-Kt]) \qquad \text{(Eq. 11)}$$

Thus a relatively simple expression describes excretion of the metabolites of acetaminophen. Equation 11 was used in the form

$$\log_e(1 - Am/Am^\circ) = -Kt \qquad \text{(Eq. 12)}$$

After the shifts in zero time, at each of the data point times except the last ones, values of K were calculated, using the experimental data and Eq. 12. The values were then averaged to give a mean value of this quantity for a given experiment. These values were then divided into 0.693 to give the rate of the processes in terms of a half-life ($t^1/_2$). Because K represents the sum of all rate constants determining the rate of removal of acetaminophen

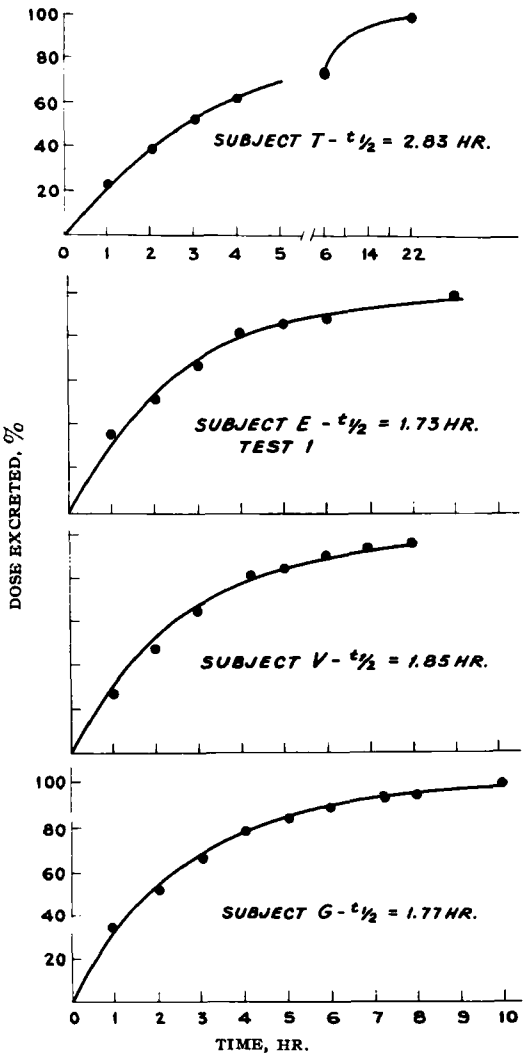


Fig. 1.—Cumulative excretion curves of acetaminophen's metabolites following oral ingestion of 1-Gm. doses of acetaminophen. Curves shown are theoretical for excretion after the time taken as zero time. Plotted points are experimentally observed values of cumulative excretion at the same times. Each curve is labeled with the test subject's designation and half-life for disposal of the drug from the body in a given experiment.

TABLE III.—RATE CONSTANTS AND HALF-LIVES FOUND FOR THE URINARY EXCRETION OF FREE ACETAMINOPHEN BY HUMANS

Test Subject	E-1	E-2	E-3	E-4	E-5	V	G	T	S	Mean
Rate constant, (k_3), hr.$^{-1}$ × 10^3	9.12	11.1	14.2	18.5	13.7	17.3	8.29	9.11	11.3	12.5
Half-life, hr.	76.0	62.4	48.8	37.4	50.6	40.0	83.6	76.1	61.3	59.6

from the body, the half-life so found represents the half-life for acetaminophen's disposal by the body.

Values of the rate constants, K, and half-lives found in all the experiments are given in Table II. To show that Eq. 12 did describe the excretion of acetaminophen's metabolites within experimental error, the mean values of K were substituted into Eq. 12 and theoretical excretion curves calculated. Some of these are shown in Fig. 1. The experimental points are included on the same plots, and it is shown that there is good agreement between theoretically predicted and experimentally observed cumulative acetaminophen metabolite excretion.

Free drug excretion was in such small amounts that it was not considered practical to fit the data to Eq. 8. The rate constant k_3 is deducible, however, from other information obtained in the experiments. At the time which corresponds to the end of the active course of experiments, Eq. 8 reduces to

$$Ae^\circ = \frac{k_3 A^\circ}{K} \qquad \text{(Eq. 13)}$$

where Ae° is the total amount of drug excreted as unchanged material. At this time Eq. 10 also holds which may be rearranged to give

$$A^\circ = \frac{KAm^\circ}{k_1 + k_2} \qquad \text{(Eq. 14)}$$

or

$$A^\circ = \frac{KAm^\circ}{K - k_3} \qquad \text{(Eq. 15)}$$

since $K = k_1 + k_2 + k_3$. If Eq. 13 is solved for A°, equated to Eq. 15, and the resulting expression rearranged, the value of the rate constant for excretion of free acetaminophen is

$$k_3 = \frac{Ae^\circ K}{Ae^\circ + Am^\circ} \qquad \text{(Eq. 16)}$$

All quantities on the righthand side of Eq. 16 are known; hence the value of k_3 may be obtained. In the present experiments the mean value was found to be 12.5×10^{-3} hr.$^{-1}$ The individual values of these constants are listed in Table III. If the relative amounts of drug conjugated with sulfuric and glucuronic acids are known, it is easy to show that the rate constants k_1 and k_2 would be, respectively

$$k_1 = \frac{Ase^\circ K}{Ae^\circ + Am^\circ} \qquad \text{(Eq. 17)}$$

and

$$k_2 = \frac{Age^\circ K}{Ae^\circ + Am^\circ} \qquad \text{(Eq. 18)}$$

where Ase° and Age° are the total amounts of drug found conjugated with sulfuric acid and glucuronic acids, respectively.

Pain Threshold Elevation and Kinetics of Metabolism.—It is of interest to compare the results of the present kinetic study with the kinetics of the analgesic effect of acetaminophen. Elevation of pain threshold following oral administration of 0.325-Gm. doses of drug to adult humans has been studied (16). After the maximum elevation in pain threshold, which occurred about 2.5 hours after administration of the doses, a rapid decay in elevation occurred. When values of elevation after the maximum were plotted as a function of time using semilogarithmic paper, a straight line resulted which indicated an apparent first-order process for decline in activity. This is shown in Fig. 2. Other instances of apparent first-order decay in pharmacological effects have been discussed by Swintosky (17). It is shown from Fig. 2 that the half-life for acetaminophen's activity is about 0.42 hours. In the paper where the data used to constant Fig. 2 were reported (16), it was stated that pain threshold elevation paralleled acetaminophen serum levels observed in another study (18). Examination of these data (which were limited) indicated that that decay in free acetaminophen levels in the post-absorptive phases had a half-life of approximately 1.9 hours. This half-life is more than four times as long as that which described decay in pain threshold elevation. The serum half-life of 1.9 hours compares favorably in magnitude to the mean half-life found in the

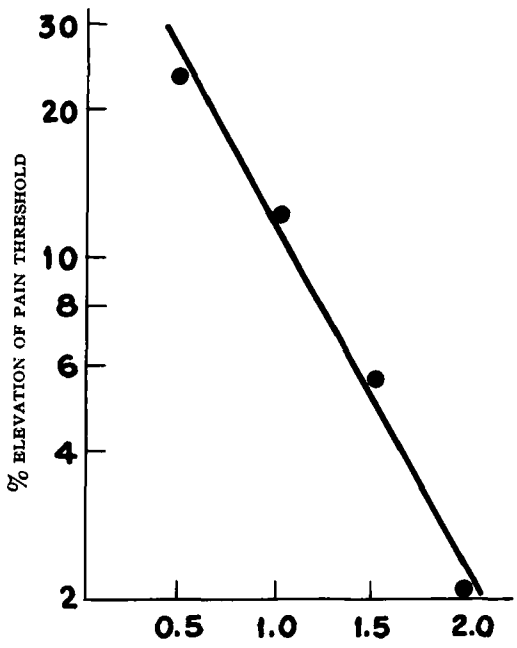


Fig. 2.—Illustrating first-order decay in pain threshold elevation after maximum observed elevation in threshold after oral ingestion of acetaminophen. Data for graph are taken from Flinn and Brodie (16).

present study of 1.95 hours. It would appear that disappearance of acetaminophen's pharmacologic effects bears little relationship to the rate with which this drug is metabolized and excreted, even though a kinetic relationship may exist between these quantities.

Biological Variations and Body Surface Area of Test Subjects.—Expected biological variation was found in the individual values of either the half-life or rate constant for disposal of acetaminophen. Since the rates of other physiological processes are sometimes related to body surface area, it was of interest to examine the present results to determine whether these were factors in causing the variation observed. There was no evidence of a relationship between surface area and rate constant when these quantities were plotted.

REFERENCES

(1) Williams, R. T., "Detoxication Mechanisms," John Wiley and Sons, Inc., New York, N. Y., 1959, p. 444.
(2) "Documenta Geigy, Scientific Tables," 5th ed., Folia Medica Geigy No. 3, Geigy Pharmaceuticals, Ardsley, N. Y., 1956.
(3) Brodie, B. B., and Axelrod, J., *J. Pharmacol. Exptl. Therap.*, **94**, 22(1948).
(4) Betts, J. J., Bray, H. G., Thorpe, W. V., and White, K., *Biochem. J.*, **61**, 4(1955).
(5) Bray, H. G., Thorpe, W. V., and White, K. I., *ibid.*, **48**, 88(1951).
(6) Bray, H. G., Humphris, B. G., Thorpe, W. V., White, K., and Wood, P. B., *ibid.*, **52**, 412(1952).
(7) *Ibid.*, **52**, 416(1952).
(8) *Ibid.*, **52**, 419(1952).
(9) Bray, H. G., Thorpe, W. V., and White, K., *ibid.*, **52**, 423(1952).
(10) Bray, H. G., Humphris, B. G., Thorpe, W. V., White, K., and Wood, P. B., *ibid.*, **59**, 162(1955).
(11) Nelson, E., and O'Reilly, I., *J. Pharmacol. Exptl. Therap.*, **129**, 368(1960).
(12) Leimieux, R. U., Sporek, K. F., O'Reilly, I., and Nelson, E., *Biochem. Pharmacol.*, **7**, 31(1961).
(13) Nelson, E., and O'Reilly, I., THIS JOURNAL, **50**, 417 (1961).
(14) Nelson, E., and O'Reilly, I., *J. Pharmacol. Exptl. Therap.*, **132**, 103(1961).
(15) Nelson, E., THIS JOURNAL, **50**, 912(1961).
(16) Flinn, F. B., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **94**, 76(1948).
(17) Swintosky, J. V., and Sturtevant, F. M., THIS JOURNAL, **49**, 685(1960).
(18) Brodie, B. B., and Axelrod, J., *J. Pharmacol. Exptl. Therap.*, **94**, 29(1948).

Aryl Indolizines I

Synthesis and Properties of Some Phenylindolizines

By VINCENT S. VENTURELLA†

Several aromatically substituted indolizines were prepared for possible psychotropic activity utilizing the Tschitschibabin method, and the susceptibility of the nucleophilic position toward benzoyl chloride was established. The properties of the prepared compounds indicated that those with an aromatic halide are not susceptible to nucleophilic attack such as the formation of alkoxy derivatives and also that those compounds containing the *p*-nitrophenyl group could be reduced to the corresponding amino compounds only under drastic conditions not normally expected of such compounds.

THE PHYSIOLOGICAL ACTIVITY of serotonin analogs (1) and reserpine (2) suggests that the structurally similar diphenylindolizines may possess psychotropic activity. In addition, it has been reported (3) that several alkylindolizines have a convulsant activity which would tend to indicate a profound effect on the central nervous system. It is hoped that the use of aryl substituents will moderate this activity thus producing compounds having the desired effect.

It is possible to prepare substituted indolizines by methods developed by Tschitschibabin (4), Barrett (5), or Scholtz (6), the former being most desirable because of ease of formation, good yields, and availability of starting materials. A modification of the method of Moser and Bradsher (7) permitted easy quaternization of the corresponding aryl pyridine with a substituted phenacyl halide to produce a solid product (Table I) in the absence of solvent followed by cyclization according to the scheme shown in Eq. 1.

When 2-parachlorobenzylpyridine was employed, the conditions necessary for the quaternization forced a spontaneous cyclization to occur, thus making it difficult to isolate these intermediates. Considerable decomposition of the tar base, which was always present in excess, was evident in the quaternization step either with 2-benzylpyridine or the *p*-chloro analog. The indolizines prepared are summarized in Table II.

The resonating system of the indolizines in the active state results in an alternating system of electron density (8) which causes nucleophile activity at position 1 or at position 3 if the former is substituted, leading to facile attack by electrophilic reagents. Thus treat-

Received December 18, 1962, from the College of Pharmacy, Fordham University, New York, N. Y.
Accepted for publication February 4, 1963.
Supported by Grant No. MY-5716, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
† Present address: Abbott Laboratories, North Chicago, Ill.