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DETERMINATION OF 7-IODO-1,3-DIHYDRO-1-METHYL-5-(2′-FLUOROPHENYL)-2H-1,4-BENZODIAZEPIN-2-ONE (Ro 7-9957) AND ITS MAJOR BIOTRANSFORMATION PRODUCTS IN BLOOD AND URINE BY ELECTRON CAPTURE–GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A sensitive and specific electron capture–gas chromatographic assay was developed for the determination of 7-iodo-1,3-dihydro-1-methyl-5-(2′-fluorophenyl)-2H-1,4-benzodiazepin-2-one (I) and its major metabolites in blood and urine. The overall recovery of I and its N-desmethyl metabolite (II) from blood is apparently quantitative. The recovery of the major urinary metabolite, the N-desmethyl-3-hydroxy analog (IV), and the minor metabolites, the N-desmethyl analog (II) and the N-methyl-3-hydroxy analog (III) added to urine as authentic reference standards ranged from 80 to 85%. The sensitivity limits of detection are of the order of 2–3 ng of I and 4–5 ng of II per ml of blood or urine. The method was applied to the determination of blood levels and the urinary excretion pattern in a dog following oral and intravenous administration of a 1-mg/kg dose (total 13 mg), and in man following the intravenous administration of single 5- and 10-mg doses. The N-desmethyl metabolite II was more predominant in dog blood than was the orally or intravenously administered I, but II was barely measurable in human blood.

INTRODUCTION

The 1,4-benzodiazepine class of compounds is clinically important because of their extensive use as tranquilizers, hypnotics and muscle relaxants[1].

The compound 7-iodo-1,3-dihydro-1-methyl-5-(2′-fluorophenyl)-2H-1,4-benzodiazepin-2-one (I) is a new member of this series[2]. It differs from diazepam in having an iodo group instead of a chloro group in position 7 and a fluoro group in position 2′ of the 5-phenyl ring (Fig. 1). The compound is of clinical interest as an anticonvulsant agent in epilepsy[3].

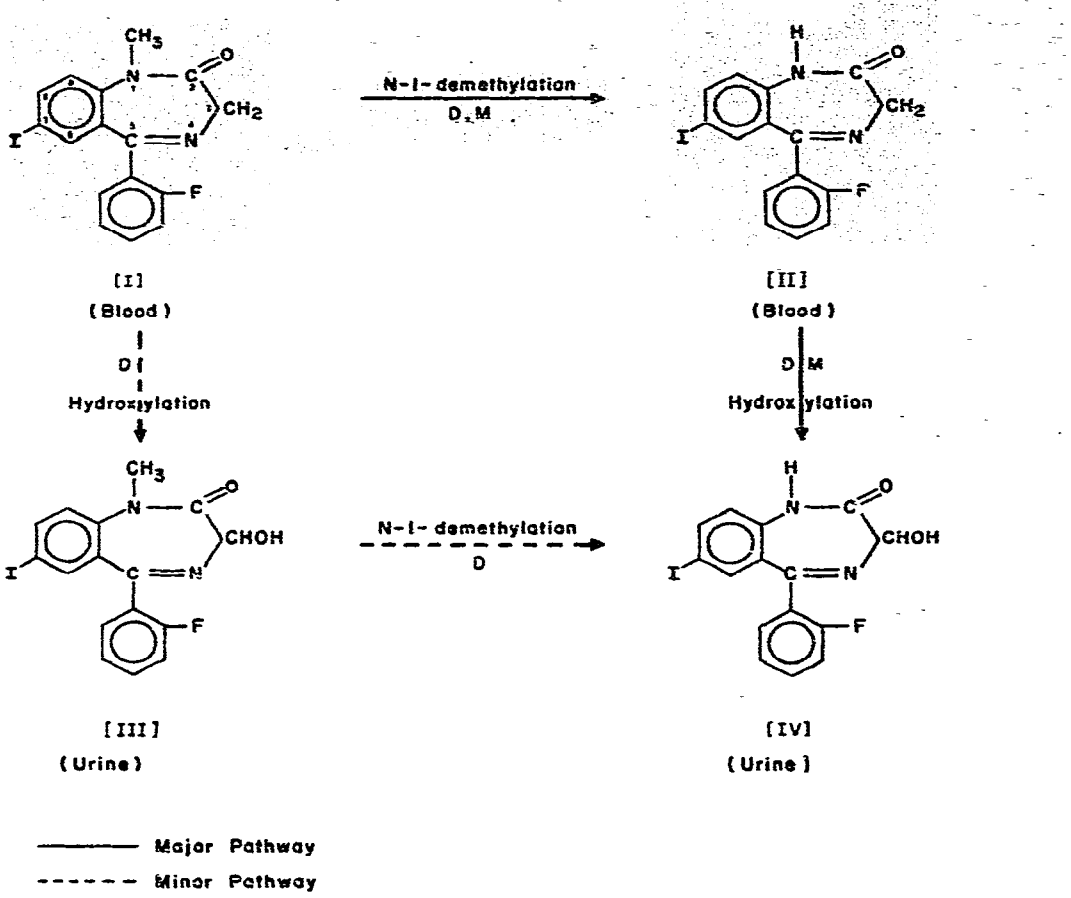

Fig. 1. Postulated pathways of the biotransformation of I in the dog (D) and in man (M) based on those reported for diazepam in these species[4,9].

Since I is analogous to diazepam its biotransformation in the dog and in man was expected to be similar to that of diazepam[4]. Authentic reference compounds of the expected metabolites were synthesized (Table I) and used to identify and quantitate the major metabolites of I in the dog and in man. It was found as expected that this compound I was rapidly and extensively metabolized producing measurable amounts of the N-desmethyl metabolite (II) in the blood and the N-desmethyl-3-hydroxy analog (IV) in the urine (Fig. 1).

The response of I and II to electron capture–gas–liquid chromatography (GLC–ECD) was sufficiently sensitive for quantitation in the nanogram range. The GLC assay reported here is a modification of that developed for medazepam and diazepam[5], and also employs the liquid phase OV-17 to resolve the intact drug I from its major metabolites in blood and urine. Diazepam is used as the reference standard and the high sensitivity of the ^{63}Ni electron capture detector (ECD) is used in the pulsed d.c. operational mode for the determination of I and its metabolites as the intact benzodiazepin-2-ones.

TABLE I

NAMES AND PHYSICAL PROPERTIES OF THE COMPOUNDS REFERRED TO IN FIGS. 1 AND 5

The 7-iodo-1,4-benzodiazepin-2-ones were synthesized by G. F. Field. The benzophenones were prepared from their respective benzodiazepin-2-ones by acid hydrolysis in 6 *N* HCl[2]. The 9-acridanones and quinazolinones were synthesized by R. I. Fryer using published procedures[10,11].

Type	*Compound*	*Chemical Name*	*Mol. wt.*	*M.p. (°C)*
1,4-Benzodiazepin-2-ones	I	7-Iodo-1,3-dihydro-1-methyl-5-(2′-fluorophenyl)-2H-1,4-benzodiazepin-2-one	395.15	107–110
	II	7-Iodo-1,3-dihydro-5-(2′-fluorophenyl)-2H-1,4-benzodiazepin-2-one	380.16	222–224
	III	7-Iodo-1,3-dihydro-3-hydroxy-1-methyl-5-(2′-fluorophenyl)-2H-1,4-benzodiazepin-2-one	410.19	188–190
	IV	7-Iodo-1,3-dihydro-3-hydroxy-5-(2′-fluorophenyl)-2H-1,4-benzodiazepin-2-one	396.16	184–187
Benzophenones	V	2-Methylamino-5-iodo-2′-fluorobenzophenone	355.16	97–100
	VI	2-Amino-5-iodo-2′-fluorobenzophenone	341.12	102–105
9-Acridanones	VII	2-Iodo-10-methyl-9-acridanone	335.15	194–196
	VIII	2-Iodo-9-acridanone	321.11	>350
Quinazolinones	IX	6-Iodo-4-(2-fluorophenyl)-1-methyl-2(1H)-quinazolinone	380.16	242–246
	X	4-(2-Fluorophenyl)-6-iodoquinazolin-2(1H)-one	366.14	>350

The method was applied to the determination of blood levels and the urinary excretion of I and its metabolites in a dog given intravenous and oral doses of 1 mg/kg, and in man following the intravenous administration of single 5- and 10-mg doses.

EXPERIMENTAL

Analysis for I and its major metabolites in blood

Parameters for GLC analysis

Column. The column packing was a pre-tested preparation containing 3% OV-17 on 60–80 mesh Gas-Chrom Q (Applied Science Labs., State Park, Pa., U.S.A.) packed in a U-shaped 4 ft. × 4 mm I.D. borosilicate glass column. The column was conditioned at 325° for 4 h with no flow of carrier gas, followed by 12 h at 275° with

carrier flowing at a rate of 40 ml/min. The useful life span of such a column was about 4–5 months of continuous use.

Instrumental parameters. A Micro-Tek Model MT-220 gas chromatograph, (Tracor, Austin, Tex., U.S.A.) equipped with a ^{63}Ni ECD containing a 15-mC ^{63}Ni β-ionization source was used. Argon–methane (9:1), oil-pumped and dry (Matheson Gas Products, East Rutherford, N.J., U.S.A.), was used as the carrier gas; the column head pressure was adjusted to 40 p.s.i.g. and the flow-rate to 120 ml/min with the detector purge gas adjusted to 20 ml/min. The temperature settings were as follows: oven 250°, injection port 280°, detector 325°. The conditions of column head pressure, flow-rate and oven temperature must be adjusted so as to obtain a retention time of 4.5 min for diazepam. Under these conditions the retention times of I and the N-desmethyl metabolite II are 8.0 and 11.4 min, respectively. A typical chromatogram is shown in Fig. 2. The solid-state electrometer (Micro-Tek, No. 8169) input was set at 10^2 and the output attenuation was 32 giving a response of 3.2×10^{-9} A for full-scale deflection (f.s.d.), the chart speed was 30 in./h, and the time constant on the 1.0-mV recorder (Model No. 194, Honeywell, Fort Washington, Pa., U.S.A.) was 1 sec (f.s.d.). The response of the ^{63}Ni ECD (operated in the pulsed d.c. mode) to I, II and the reference standard diazepam showed maximum sensitivity at 50 V d.c.

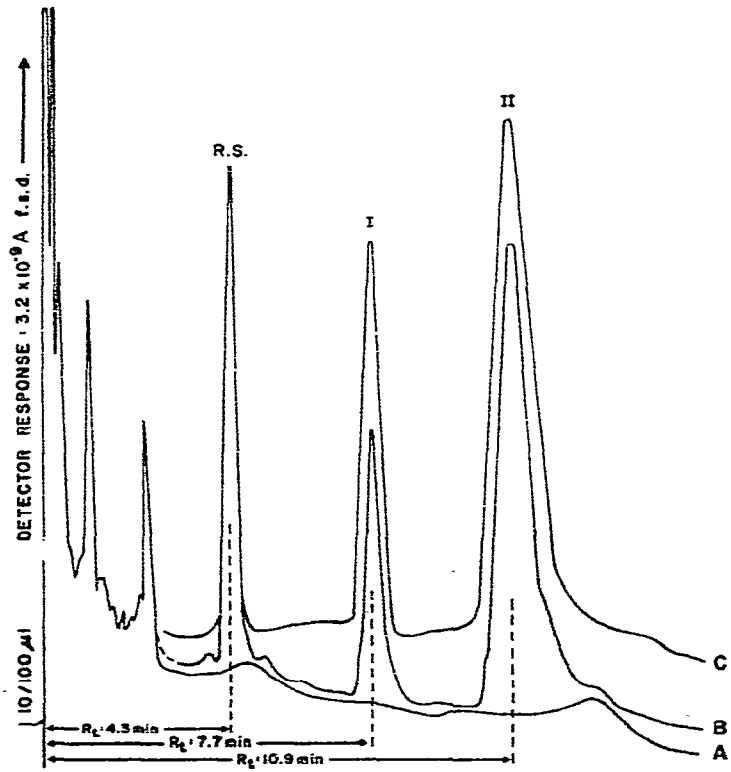

Fig. 2. Gas chromatograms of benzene–methylene chloride (9:1) extracts of A, control dog blood; B, control dog blood containing added authentic standards; C, dog blood post intravenous administration of I. R_t = retention time; R.S. = reference standard (diazepam).

with a 270-μsec pulse rate and a 4-μsec pulse width. Under these conditions 1.0 ng of I and 3 ng of diazepam give nearly full-scale deflection on the 1.0-mV recorder. The minimum detectable amount of I is 1–2 ng/ml of blood.

Assay in blood

Preparation of standard solutions. The respective benzodiazepin-2-ones that are required as analytical standards are listed in Table I. Weigh out 10.00 mg each of the free base of I and its N-desmethyl metabolite II into separate 10-ml volumetric flasks. Dissolve I in 1.0 ml of absolute methanol followed by 1 ml of absolute ethanol and make up to volume with acetone–*n*-hexane (1:4). Dissolve II in 2 ml of acetone and make up to volume with *n*-hexane. Prepare a stock solution of diazepam (10.00 mg) as described for I. These stock solutions A contain 1 mg/ml. Prepare working standard solutions B in acetone–*n*-hexane (1:4) containing a mixture of the authentic standards I and II and the reference standard (diazepam) in the following concentrations in separate 10-ml volumetric flasks as indicated in Table II.

TABLE II

CONCENTRATIONS OF STANDARD SOLUTIONS FOR ASSAY IN BLOOD

Figures in parentheses represent concentrations (ng per 10 μl) injected for GLC analysis.

Standard solution	*Concentration (ng/ml)*		
	I	*II*	*Diazepam*
B_1	25 (0.25)	50 (0.5)	300 (3.0)
B_2	50 (0.50)	100 (1.0)	300 (3.0)
B_3	75 (0.75)	150 (1.5)	300 (3.0)
B_4	100 (1.0)	200 (2.0)	300 (3.0)

Aliquots of solutions B_1 to B_4 (10 μl) are injected into the chromatograph to establish the ECD response to the compounds and the parameters for GLC analysis. Additionally, 100-μl aliquots of solutions B_1 to B_4 are added to blood as the internal standards to monitor the relative recovery with respect to the reference standard (diazepam).

The recovered internal standards are used to prepare a calibration curve (Fig. 3) for the quantitation of the concentrations of I and II in biological specimens. A new calibration curve has to be made with each set of unknowns.

Reagents. All reagents must be of analytical reagent grade (> 99% purity) and all inorganic reagents are made up in distilled de-ionized water. These include 1 *M* H_3BO_3–Na_2CO_3–KCl buffer (pH 9.0) prepared as previously described[5] and normal saline (0.9% NaCl in water). The organic solvents used are benzene and methylene chloride (nanograde) (Mallinckrodt, St. Louis, Mo., U.S.A.). Acetone is pesticide grade and *n*-hexane is 99 mol% pure "H-301" (Fisher, Pittsburgh, Pa., U.S.A.). A mixture of benzene–methylene chloride (9:1) is used as the solvent for

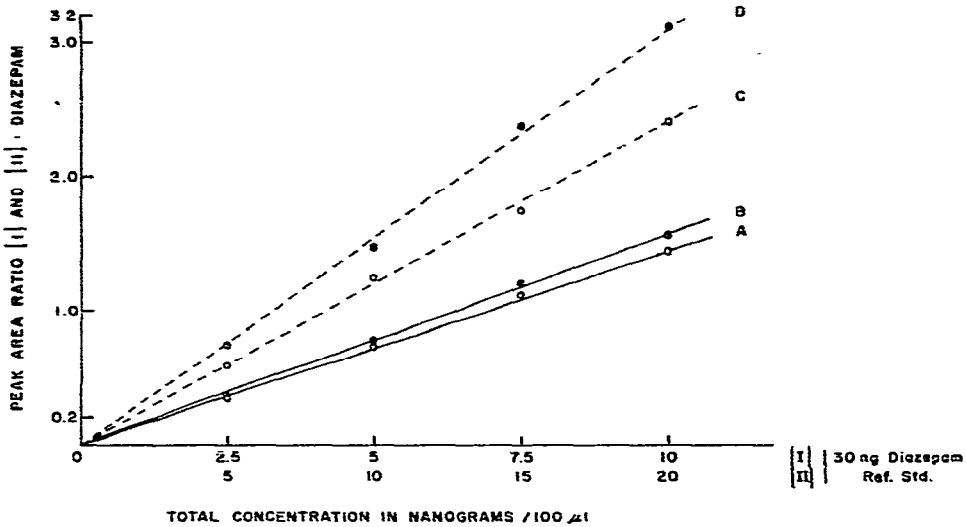

Fig. 3. ECD calibration curves of I, and its major blood metabolite II. A and B, external standard and internal standard curve of I, respectively. C and D, external and internal standard curve of II, respectively.

extraction, and a mixture of acetone–*n*-hexane (1:4) is used as the solvent for GLC–ECD analysis.

Procedure. Into a 15-ml conical centrifuge tube containing 30 ng of diazepam (added as a reference standard) add 1.0 ml of whole blood, 1 ml of normal saline solution (0.9% NaCl in water), 3 ml of borate buffer (pH 9.0) and mix well on a Vortex action mixer for 30 sec. Then add 8 ml of benzene–methylene chloride (9:1) for extraction. Along with the samples process a specimen of control blood (taken preferable from the subject prior to medication) and four 1-ml specimens of control blood to which 100 µl of solutions B_1, B_2, B_3 or B_4 are added to comprise a set of internal standards. Seal the tubes with PTFE stoppers and extract by shaking for 10 min on a reciprocating shaker (Eberbach, Ann Arbor, Mich., U.S.A.) at about 80–100 strokes per min. Centrifuge the samples for 10 min at 8–10° in a refrigerated centrifuge (Model PR-J with a No. 253 rotor; Damon/IEC Division, Needham, Mass., U.S.A.) at 2600 rpm (1500 *g*). Transfer a 7-ml aliquot of the supernatant into a fresh 15-ml centrifuge tube and evaporate to dryness at 60° in the water-bath of a N-EVAP Model No. N-07 evaporator (Organomation Assoc., Worchester, Mass., U.S.A.) under a stream of pure dry nitrogen. Vacuum dry the residue (over Drierite) in a vacuum desiccator for 10 min to remove all traces of moisture, dissolve the residue in 100 µl of acetone–*n*-hexane (1:4) and inject a suitable aliquot (5–10 µl) for EC–GLC analysis. The above method is limited to the analysis of 1 ml of whole blood or less. If greater sensitivity is required, then up to 4 ml of blood can be extracted with diethyl ether using a procedure which requires extensive clean up, details of which have been published[6].

The peaks due to the intact drug I, its metabolite II and the reference standard are identified by their respective retention times (Fig. 2), and their respective peak

areas are determined either by measuring peak height (cm) × width at half-height (cm) using the slope baseline technique or by electronic digital integration.

Calculations. The peak area ratios of I and II to diazepam in the aliquots of the unknowns injected are used to interpolate the concentrations directly from the respective internal standard curves (Fig. 3, curves B and D). The sensitivity of the GLC–ECD assay is of the order of 2–3 ng of I and 4–5 ng of II per ml of blood.

If further dilution of the unknown sample (*i.e.*, $< 100\ \mu l$) is necessary because of high levels of I and II, the reference standard may be diluted to such an extent that the peak area ratio technique becomes impractical. In this event direct calibration has to be employed whereby calibration curves of peak area of the recovered internal standards of I and II *vs.* concentration are plotted and used for the quantitation of the unknowns. Furthermore, the amounts of I and II per aliquot of the unknown sample injected have to be corrected for the dilution of the total sample.

Assay in urine

Preparation of standard solutions. Prepare a working solution C containing a mixture of 3.0 μg II, 25 μg III and 10 μg IV in 10 ml of acetone–*n*-hexane (1:4) by combining the appropriate aliquots of their stock solutions (A). A 10-μl volume of solution C contains 3.0 ng II, 25 ng III and 10 ng IV. Inject suitable aliquots of solution C into the gas chromatograph to establish the ECD calibration curves for each compound, and 100-μl aliquots are added to urine as internal standards for the quantitation of these metabolites.

Procedure. The sample preparation and extraction procedure used is similar to that described for diazepam[5]. Into a 50-ml erlenmeyer flask add 5.0 ml urine, 5 ml phosphate buffer (pH 5.3) and 0.1 ml of Glusulase (1% of total volume). Along with the samples run a 5-ml specimen of control urine (taken preferably from the subject prior to medication) and duplicate 5-ml specimens of control urine containing 100 μl of C equivalent to 100 ng of IV, 30 ng of II and 250 ng of III added as internal standards. The samples are then incubated and processed exactly as described for diazepam[5] with one exception. The 6.0 *N* HCl extract must be neutralized carefully in an ice-bath by the dropwise addition of 6.0 *N* NaOH to prevent excessive heat of neutralization which could partially hydrolyze the compounds to their benzophenones. The sample is titrated to pH 9.0 using a pH meter. This is necessary to effect optimal extraction of the N-desmethyl metabolites II and IV.

Quantitation of the urinary metabolites. The presence of several metabolites in the urine results in a complex chromatogram (Fig. 4). Therefore, the direct calibration technique has to be used for quantitation. The analysis of a 5–10-μl aliquot usually results in a peak for IV which is off-scale thus necessitating further dilution prior to re-injection. The peaks for the N-desmethyl analog II and the N-methyl-3-hydroxy analog III, however, are on-scale and can be directly quantitated. The amount of each urinary component is calculated directly by comparison of the peak areas to that of the respective internal standards[5].

RESULTS AND DISCUSSION

Compounds I and II were quantitatively extracted from blood buffered to pH 7.0 and 9.0. Extraction of blood buffered to pH 9 with borate buffer gave "cleaner"

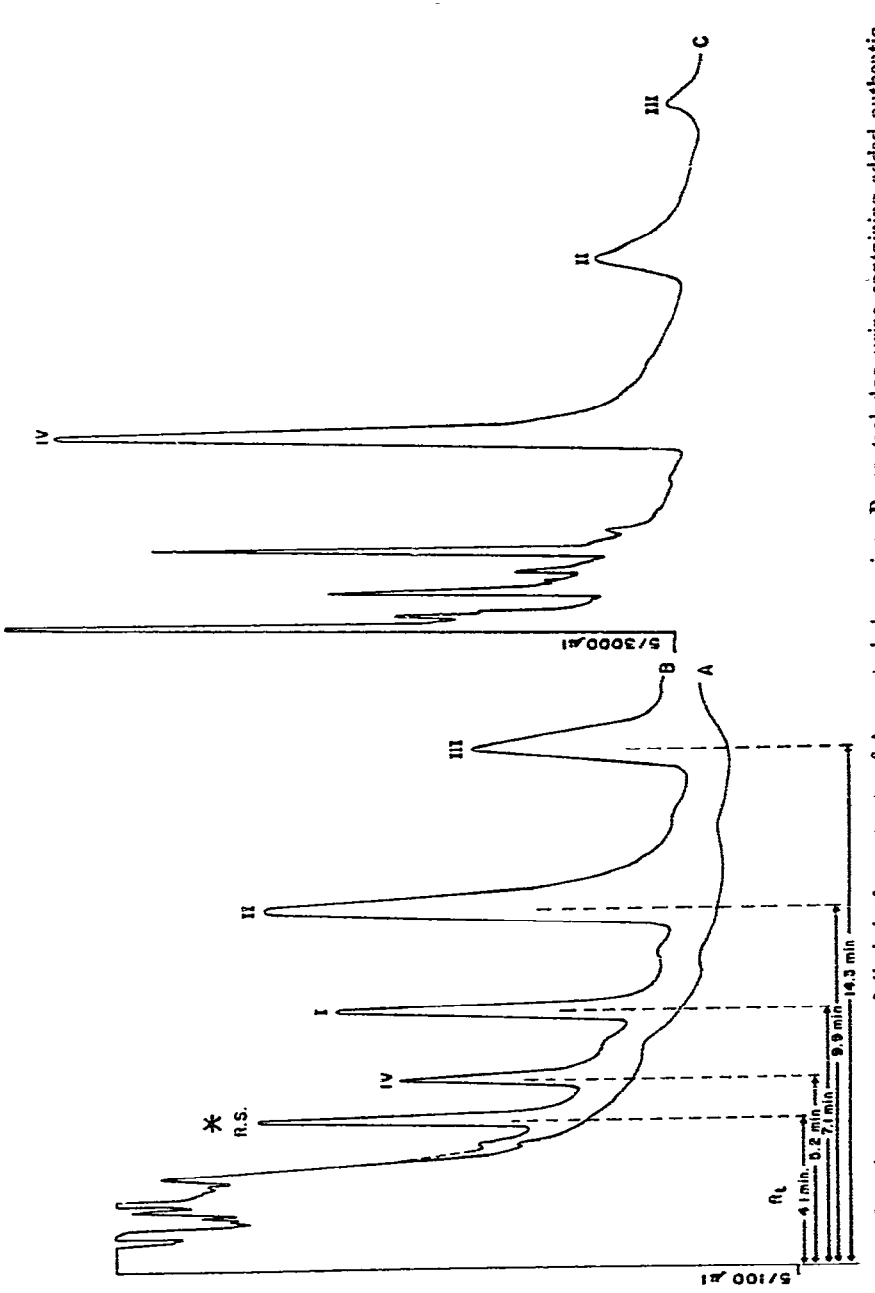

Fig. 4. Gas chromatograms of diethyl ether extracts of A, control dog urine; B, control dog urine containing added authentic standards; C, dog urine (0–24 h) post medication (intravenous or oral). R_t = retention time. * R.S. = reference standard (diazepam).

control chromatograms by GLC analysis and was preferred. When authentic standards of I and II in acetone–*n*-hexane (1:4) were analyzed by GLC, the two components were well resolved, the peak due to I was very symmetrical, whereas that due to II showed tailing. The chromatograms obtained from the addition of both compounds to either control blood or to 6 *N* HCl in the absence of blood were significantly different. By the former procedure, the peak due to I was eluted at the same retention time as before, but its sensitivity to the EC detector was enhanced. The peak due to II not only showed greater sensitivity to the ECD, but it was also eluted as a sharper, Gaussian-shaped peak with a retention time which was shorter by about 1 min than that of authentic II. By the latter procedure, the same observations were noted but to a lesser extent. These phenomena indicated the formation of either a chemical derivative or an adsorption complex, especially in the presence of lipids extracted from blood. Consequently, the recovery of I and II from blood when determined against an external standard curve of the pure authentic compounds I and II is greater than 100%; mean, 106% ± 5.0 for I and 112% ± 5.0 for II (Fig. 3, curves A and C). It is possible that the extracted lipids either interact with exposed active sites on the column and reduce surface adsorption effects through hydrogen bonding or form an adsorption complex with the amide bond $-\overset{H}{\underset{|}{N_1}}-\overset{O}{\overset{\|}{C_2}}-$ of the N-desmethyl metabolite II, thus minimizing its adsorption on the column. The use of internal standards of I and II added in varying concentrations to blood and taken through the entire procedure is essential for obtaining valid quantitation of I and II in unknown specimens.

The known chemical reactions and derivatives of I and II are shown in Fig. 5. Both compounds undergo hydrolysis in strong acid at 100° to their respective benzophenones which can be cyclized in dimethylformamide (DMF) in K_2CO_3 at 100° to the 9-acridanone derivatives[7]. Furthermore, the N-desalkyl-1,4-benzodiazepin-2-ones undergo thermolytic rearrangement during GLC analysis to form quinazolines[8] which could be responsible for the anomalous behavior of II. Authentic reference standards of the quinazolinones IX and X were synthesized and used as analytical standards to test this hypothesis. It was found that neither the respective benzophenones, the 9-acridanones, nor the quinazolinones were the derivatives formed during GLC analysis. Consequently, the chemical nature of the derivatives and/or complexes formed has yet to be elucidated.

The determination of the actual percentage recovery is not necessary for purposes of quantitation on a routine basis, because the internal standards taken through the entire assay give valid analytical data since they automatically compensate for these anomalies and for the recovery of both components in the unknowns. The added reference standard diazepam (whose recovery is > 95%) serves as the index of any procedural variation incurred in sample processing.

Application of the method in biological specimens

Studies in the dog. The blood levels and the urinary excretion of I and its major metabolites were determined in a dog following the administration of a single 1-mg/kg dose (total 13 mg) by intravenous (I.V.) and oral routes. The blood level curves are shown in Fig. 6. Following I.V. administration the intact drug I was measurable for

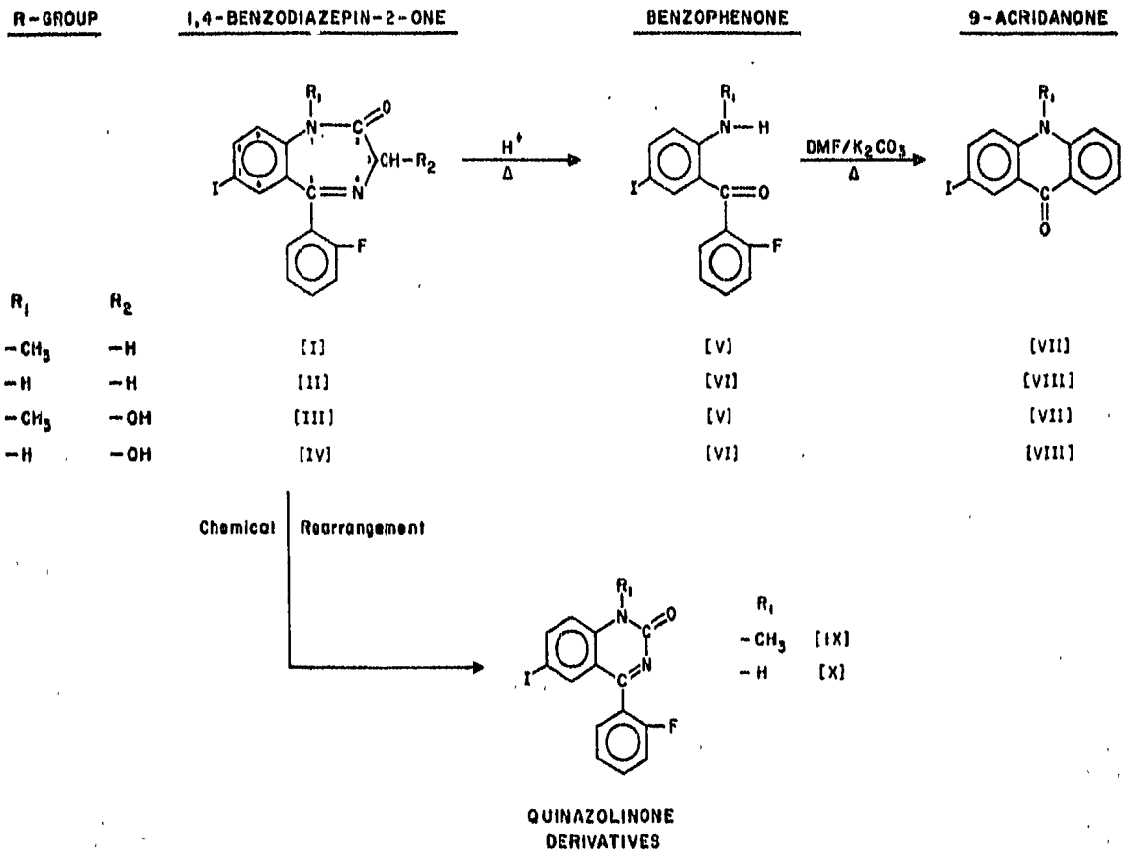

Fig. 5. Reactions of 1 and its major metabolites.

up to 24 h. The concentration of I declined very rapidly from a peak level of 1.3 μg/ml at 1 min to threshold levels at 12 and 24 h. The N-desmethyl metabolite II was seen almost immediately after the I.V. administration of I; its levels reached a peak (0.40 μg/ml) at about 10 min and declined progressively with time with a half-life of about 6 h.

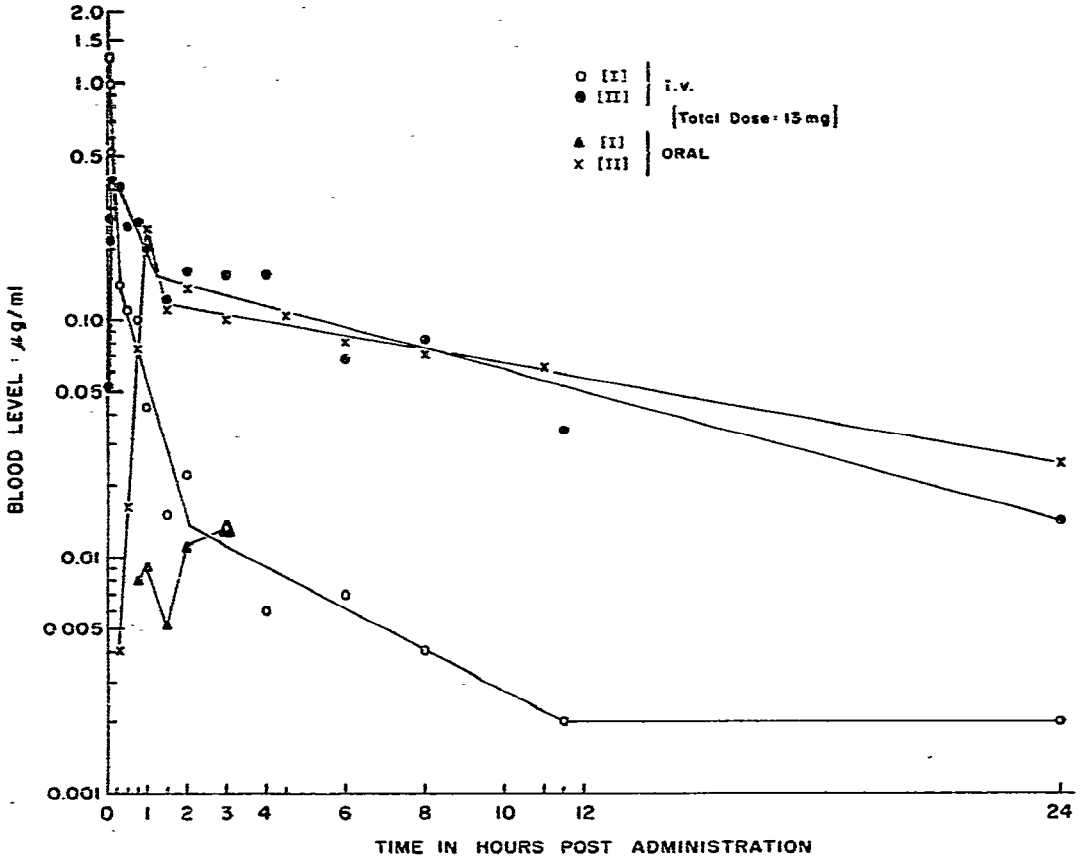

Fig. 6. Blood level fall-off curves of I and its N-desmethyl metabolite II following a single 1 mg/kg dose by oral and intravenous (i.v.) routes in the dog.

Following oral administration, the intact drug was seen in very low amounts from 45 min to 3 h post dosing whereas II was again the major blood component. It was measurable at 15 min, reached a peak at 1 h (0.25 μg/ml) and declined progressively with time, with a half-life of about 10 h. The blood levels of II resulting from the i.v. or oral administration of I were very similar indicating both rapid absorption of I and rapid biotransformation to II.

The urinary excretion data from specimens collected over a 48-h period in the same animal are shown in Table III. The major metabolite was the N-desmethyl-3-hydroxy analog IV which accounted for 3.83% of the dose following i.v. and 4.11%

TABLE III

URINARY EXCRETION DATA ON I AND ITS METABOLITES IN A DOG

N.M. = Not measurable: <0.002 μg I, <0.004 μg II, <0.050 μg III, <0.020 μg IV, per ml of urine.

Dose	*Route*	*Excretion period (h)*	*Excreted (μg)*			
			I	*II*	*III*	*IV*
1 mg/kg (13 mg)	Intravenous	0–24	N.M.	2.8	8.3	385
		24–48	N.M.	1.7	N.M.	113
		Total	—	4.5	8.3	498
		% of dose excreted	—	0.04	0.06	3.83
1 mg/kg (13 mg)	Oral	0–24	N.M.	3.4	11.1	408
		24–48	N.M.	N.M.	N.M.	126
		Total	—	3.4	11.1	534
		% of dose excreted	—	0.03	0.08	4.11

of the dose following oral administration. No measurable amounts of the intact drug I were seen in any of the urine specimens. The N-desmethyl analog II and the N-methyl-3-hydroxy analog III each accounted for less than 0.1% following i.v. and oral administration. The low amounts of the metabolites recovered suggest either extensive tissue distribution and/or an alternate route of elimination.

Characterization of the blood and urinary metabolites in the dog by thin-layer chromatography. Two-dimensional thin-layer chromatography (TLC) was used to confirm the identity of I and its metabolites seen in the dog. The acetone–*n*-hexane solutions (remaining after GLC analysis) of the diethyl ether extracts of blood and urine following oral dosing were pooled, concentrated by evaporation and transferred onto Merck F_{254} pre-coated silica gel G/F chromatoplates. The plates were developed in vapor saturated chambers (lined with Whatman No. 1 paper) in two dimensions using chloroform–heptane–ethanol (5:5:1) in the first dimension and heptane–ethyl acetate–ammonia (50:50:1) in the second dimension. Authentic standards of I, II, III and IV were run as markers along the margins.

The chromatogram of the pooled blood specimen representing 26 ml showed the presence of only the intact drug I and its N-desmethyl analog II as ultraviolet (UV) absorbing spots when viewed under a short-wave UV lamp (254 nm), thus confirming the components seen by GLC–ECD.

The chromatogram of the pooled urine specimen following i.v. and oral administration representing 10 ml each was run in one dimension in chloroform–heptane–ethanol (5:5:1) and showed the presence of trace amounts of the N-desmethyl metabolite II, and the N-methyl-3-hydroxy metabolite III. The major component was the N-desmethyl-3-hydroxy analog IV, again confirming the GLC findings.

Studies in man. Four healthy adult volunteers, three males and one female ranging in age from 21 to 51 years with no previous history of benzodiazepine therapy were selected. Two subjects (V. J. and S. S.) were administered a single 5 mg i.v. dose of I while the other two (K. K. and S. K.) were administered a single 10 mg i.v. dose of I. Blood specimens were drawn prior to administration (0-h control),

TABLE IV

BLOOD LEVELS (μg/ml) OF I AND ITS N-DESMETHYL METABOLITE II IN 4 PATIENTS FOLLOWING INTRAVENOUS ADMINISTRATION OF SINGLE 5- AND 10-mg DOSES

Doses = patient V.J., 5 mg = 0.05 mg/kg; S.S., 5 mg = 0.082 mg/kg; K.K., 10 mg = 0.116 mg/kg; S.K., 10 mg = 0.120 mg/kg. Ages: V.J. (female), 51 year; S.S (male), 21 year; K.K. (male), 21 year; S.K. (male), 23 year. N.M. = Not measurable: <0.001 μg/ml for I and <0.002 μg/ml for II. N.S.T. = No sample taken.

Time	*V.J.*		*S.S.*		*K.K.*		*S.K.*	
	I	*II*	*I*	*II*	*I*	*II*	*I*	*II*
0 h	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.
5 min	0.59	N.M.	0.240	N.M.	1.54	0.002	2.56	0.004
10 min	0.30	N.M.	0.170	N.M.	0.51	0.003	0.47	0.004
15 min	0.18	N.M.	0.150	0.002	0.36	0.003	0.41	0.004
20 min	0.10	N.M.	0.130	0.002	0.35	0.004	0.23	0.005
25 min	0.09	N.M.	0.120	0.002	0.26	0.003	0.19	0.005
30 min	0.06	N.M.	0.100	0.002	0.24	0.003	0.18	0.005
45 min	0.05	N.M.	0.089	0.002	0.20	0.004	0.17	0.005
1 h	0.05	N.M.	0.066	0.002	0.20	0.004	0.16	0.005
1.5 h	0.04	N.M.	0.062	0.002	0.14	0.004	0.15	0.006
2 h	0.03	N.M.	0.054	0.002	0.13	0.004	0.13	0.006
3 h	0.02	N.M.	0.058	0.003	0.11	0.004	0.11	0.006
4 h	N.S.T.	N.S.T.	N.S.T.	N.S.T.	N.S.T.	N.S.T.	0.13	0.007
6 h	N.S.T.	N.S.T.	N.S.T.	N.S.T.	N.S.T.	N.S.T.	0.18	0.006
24 h	0.04	0.004	0.086	0.012	0.13	0.015	0.09	0.017

and at the specific time points as shown in Table IV. A control urine specimen and a 0–24 post dosing specimen were also collected from each subject.

The blood level data on the four subjects are given in Table IV and plotted graphically in Figs. 7 and 8, respectively. Measurable blood levels of the intact drug were seen in all four subjects at the first sampling time point of 5 min after administration and were measurable throughout the 24-h experimental period. The blood level curves of I showed either a biphasic or triphasic fall-off pattern with a secondary peak seen at 6–24 h. The initial phase was seen in the 0–30 min period with apparent half-lives ranging from 5 to 18 min, while the second phase seen in the 0.5–3-h period in the four subjects indicated apparent half-lives ranging from 1.5 to 3.5 h during this time period. Additional blood samples were drawn at 3 and 6 h from the fourth subject (S. K.) and an increase in the blood levels of I was seen at these times. Therefore, in the other three subjects the blood levels of I may also have increased between 3 and 24 h. This rise may reflect recirculation from tissue storage depots. In sharp contrast to the dog, in man the N-desmethyl metabolite II was seen in trace amounts in the blood ranging from 2 to 7 ng/ml in three out of the four subjects over virtually the entire experimental period. It is interesting to note that the levels of this metabolite at the 24-h point were higher than at any other point in time.

The 0–24 h urinary excretion of I and its major metabolites in these four subjects are given in Table V in terms of total amount (μg) excreted, comprising the "free" or directly extractable fraction represented by I and II and the "bound" or glucuronide-sulfate conjugated fraction represented by the 3-hydroxy analogs III and IV. The latter metabolites are extractable only after enzymatic deconjugation with

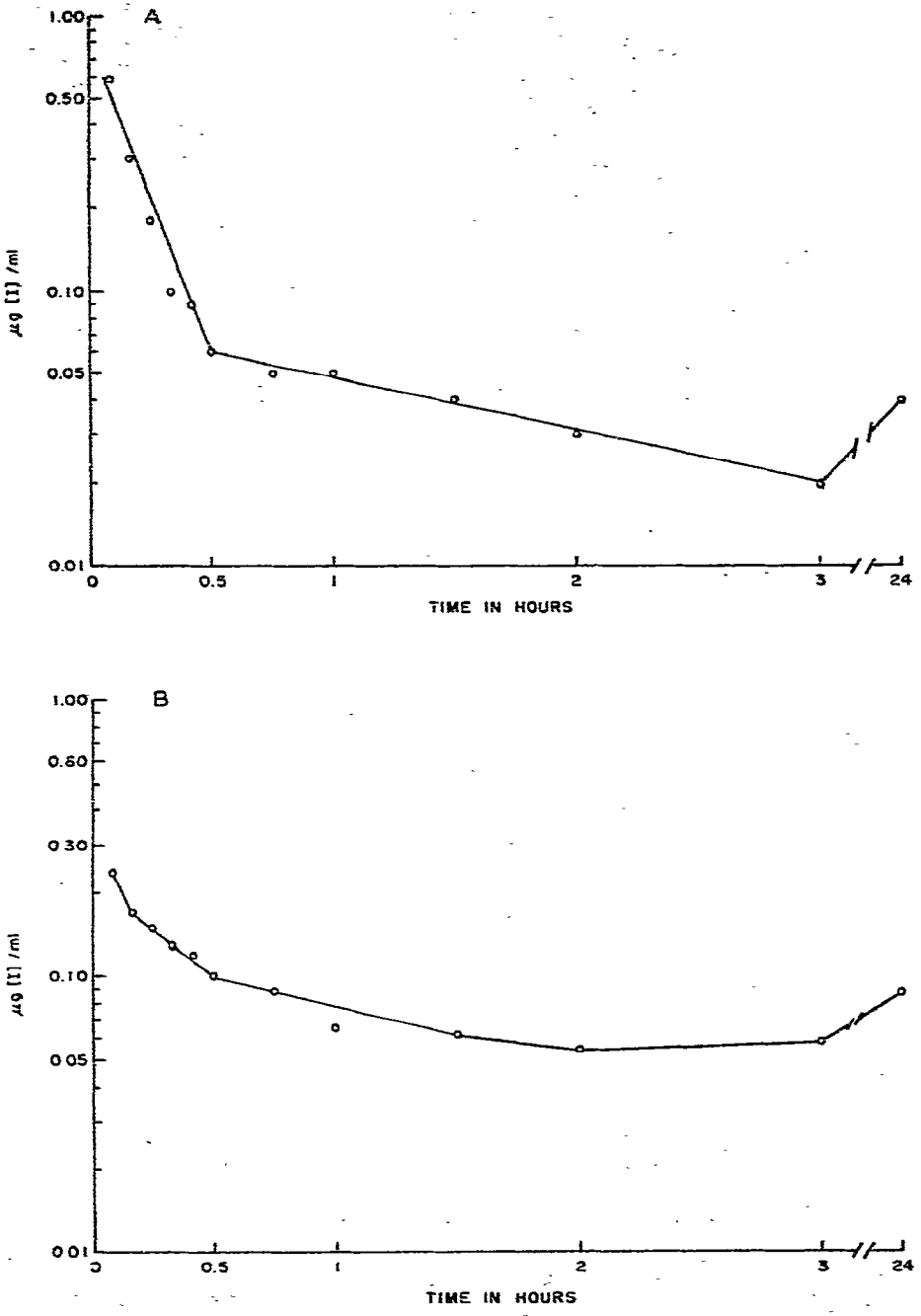

Fig. 7. Blood level fall-off curves of I in (A) patient V.J. and (B) patient S.S. following the intravenous administration of a single 5-mg dose.

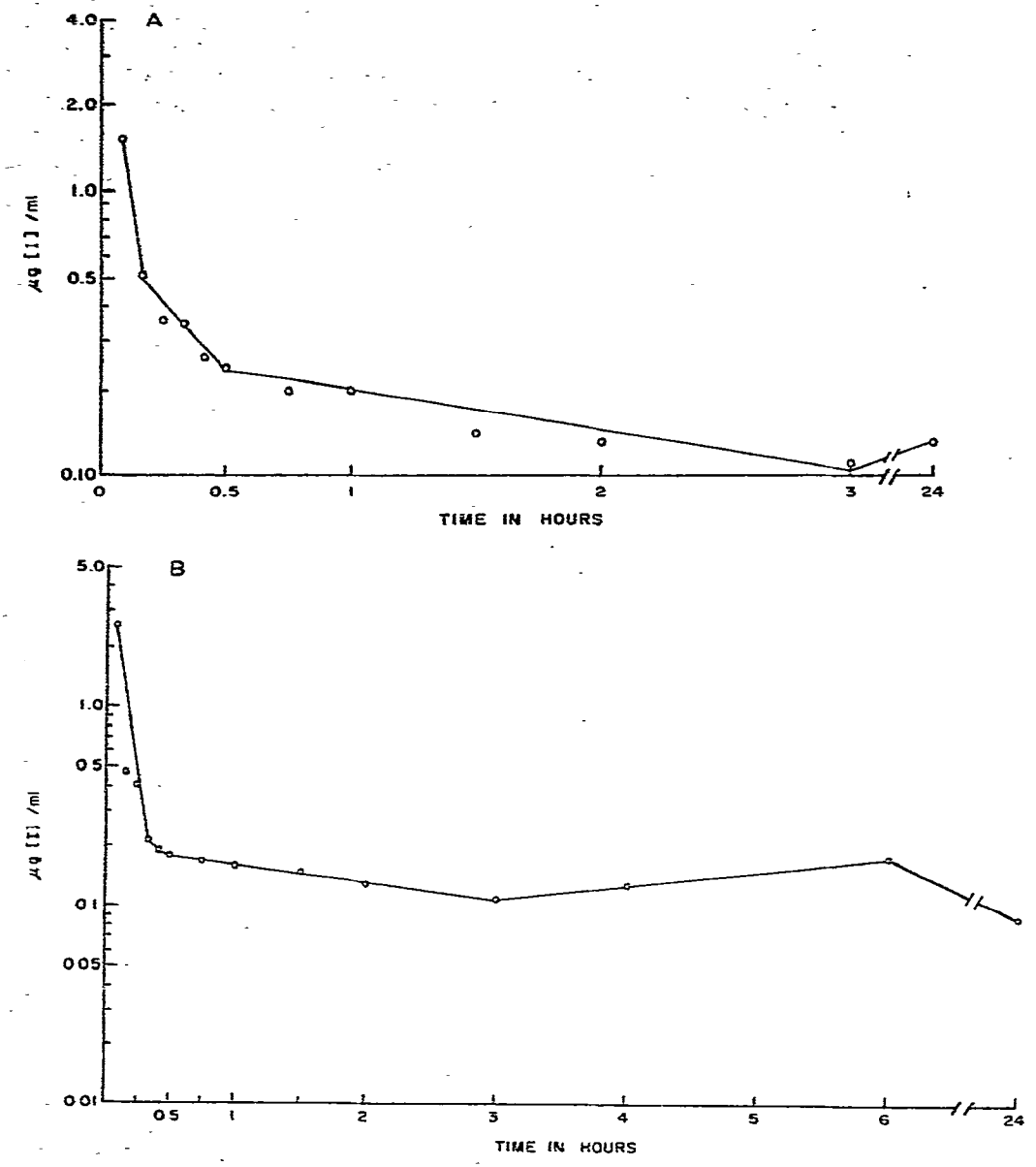

Fig. 8. Blood level fall-off curves of I in (A) patient K.K. and (B) patient S.K. following the intravenous administration of a single 10-mg dose.

Glusulase®. The total percent of the administered dose recovered was quite low and ranged from 0.18 to 1.94% in the four subjects. No measurable amounts of the intact drug or the N-methyl-3-hydroxy analog III were seen in any subject. The major metabolites recovered were the N-desmethyl analog II and the N-desmethyl-3-hydroxy analog IV present in an approximately 1:2 ratio respectively in three out of four subjects and in a 1:1 ratio in the fourth subject.

TABLE V

URINARY EXCRETION DATA ON I AND ITS METABOLITES IN 4 PATIENTS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF SINGLE 5- AND 10-mg DOSES

Collection period: 0–24 h (total volume voided). N.M. = Not measurable: <0.001 μg/ml for I and <0.050 μg/ml for III.

Patient	*Dose*	*Total excreted (μg)*				
		*I**	*II**	*III***	*IV***	*% of dose recovered*
V.J.	5 mg (765 ml)	N.M.	4.59	N.M.	4.59	0.18
S.S.	5 mg (1230 ml)	N.M.	30.75	N.M.	66.42	1.94
K.K.	10 mg (1495 ml)	N.M.	28.41	N.M.	73.26	1.02
S.K.	10 mg (864 ml)	N.M.	29.38	N.M.	58.75	0.88

* "Free" or directly extractable compounds.

** "Bound" or conjugated compounds.

The data indicate that the biotransformation pathway of I in man and in the dog is analogous to that of diazepam in these two species[4,9]. The compound appears to undergo N-demethylation to II more rapidly in the dog than in man based on the blood levels of II in both species. Hydroxylation in the C-3 position of the benzodiazepine ring results in the oxazepam-like analog IV which is excreted in the urine of both species as a conjugate. Hydroxylation in the C-3 position to produce III without either prior or subsequent N-demethylation appears to be negligible in man and only a minor route in the dog.

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