however, the authors have not found any published reports of kidney metabolism studies. This is an area that should be investigated.

The amount of aflatoxin B_1 fed to the steer in this study is considerably higher than the amount fed in other cattle studies (1-3). However, if a 160-kg calf, which would normally consume 2½-3% of its weight in feed/day (4-4.8 kg), ate contaminated feed at 500 ng/g, detectable levels of B_1 and M_1 should be found in tissues with currently available methods. This presumes that toxin transmission to tissue is proportional to the toxin ingested after a certain level of aflatoxin has been reached. According to the data of Keyl and Booth (4), aflatoxin is detrimental to cattle at a concentration between 300 and 700 ppb. Consequently, measurable quantities of aflatoxins are probably transmitted to the meat at those levels, also. It is obvious that there is still much information to be learned about the transmission of aflatoxin to edible tissues of large animals such as cattle.

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REFERENCES

1. Rodricks, J.V., and L. Stoloff, in "Mycotoxins in Human and Animal Health," edited by J.V. Rodricks, C.W. Hesseltine and M.A. Mehlman, Pathotox Publishers, Inc., Park Forest South, IL, 1977, pp. 67-79.
2. Stoloff, L., in "Interactions of Mycotoxins in Animal Production," proceedings of a symposium jointly sponsored by American Society of Animal Science, American Dairy Science Association, and Committee on Animal Nutrition/National Research Council, at Michigan State University, July 1978, pp. 157-166.
3. Edds, George T., in "Conference on Mycotoxins in Animal Feeds and Grains Related to Animal Health," sponsored by Bureau of Veterinary Medicine/Food and Drug Administration at FDA, Rockville, MD, June 1979, pp. 80-164.
4. Keyl, A.C., and A.N. Booth, JAOCS 48:599 (1971).
5. Jemmali, M., and T.R.K. Murthy, Z. Lebensm. Unter-Forsch. 161:13 (1976).
6. Trucksess, M.W., and L. Stoloff, J. Assoc. Off. Anal. Chem. 62:1080 (1979).
7. van Egmond, H.P., W.E. Paulsch, E.A. Sizoo and P.L. Schuller, Fourth International IUPAC Symposium on Mycotoxins & Phycotoxins, Lausanne, Switzerland, 1979, abstr.
8. Stubblefield, R.D., and O.L. Shotwell, J. Assoc. Off. Anal. Chem. (in press).
9. Tuite, J., in "Interactions of Mycotoxins in Animal Production," proceedings of a symposium jointly sponsored by American Society of Animal Science, American Dairy Science Association, and Committee on Animal Nutrition/National Research Council, at Michigan State University, July 1978, pp. 19-39.
10. Stoloff, L., and M.W. Trucksess, J. Assoc. Off. Anal. Chem. 62:1361 (1979).
11. Richard, J.L., J.R. Thurston, E.B. Lillehoj, S.J. Cysewski and G.O. Booth, J. Am. Vet. Res. 39:163 (1978).
12. van Egmond, H.P., and R.D. Stubblefield, J. Assoc. Off. Anal. Chem. 64:152 (1981).

Hepatotoxicity of the Mycotoxin Penicillic Acid: A Pharmacokinetics Consideration

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ABSTRACT

The hepatotoxicity of penicillic acid (PA), a carcinogenic mycotoxin, was substantiated by a variety of hepatic functional tests. Involvement of an active metabolite as the toxic species was proposed. The toxicity of PA was dependent on the route of administration with intraperitoneal (ip) being the most toxic followed by intravenous (iv) and oral. This difference in toxicity was explained by the kinetic data for PA if liver were assumed to be the site of activation. One-, 2- and 3-compartment open models were proposed to fit the plasma parent compound concentration after oral, ip, and iv administration of PA. Liver, kidneys, heart, lungs and spleen contained more radioactivity than brain, fat and muscle after [^{14}C]-PA administration. Only a fraction of the radioactivity in the blood was detected as the parent compound. Most of the recovered radioactivity in the kidneys and liver was in the cytosol fraction. [^{14}C]PA was readily metabolized in the liver. The metabolites were excreted in the bile and effectively cleared by the kidneys. Fecal and respiratory CO_2 were minor excretory routes. Over 90% of the urinary and 99% of the biliary metabolites were not extracted with polar organic solvents. Three water-soluble metabolites (derived from GSH or cysteine) were resolved by HPLC in urine and bile. About 10% of the urinary metabolites were detected as glucuronide conjugates. These data supported the hypothesis that an active metabolite which can be detoxified by GSH is involved in the toxicity of PA.

INTRODUCTION

Penicillic acid (PA), an α,β-unsaturated conjugated lactone, is produced by several food-borne fungi (1). Since the discovery of its carcinogenicity in experimental animals (2-4) and its subsequent isolation from agricultural products (5-9), PA has been considered a potential environmental health hazard to man. In addition to being carcinogenic, PA is hepatotoxic in experimental animals. For example, PA caused generalized hepatocyte necrosis in mice (10) and sinusoidal congestion in caine liver (11). Morphologic damage also occurred in hepatocytes treated with PA concentration as low as 1×10^{-5} M (12). The hepatotoxicity of PA was substantiated by studies which showed that PA increased pentobarbital-induced sleeping time while decreasing in vivo and in vitro pentobarbital metabolism (13); decreased hepatic reduced glutathione (GSH), elevated serum transaminase and bilirubin, and increased sulfobromophthalein and indocyanine green retention (14); and depressed hepatic biliary excretion of indocyanine green (15). The involvement of an active metabolite and the protective role of GSH in PA hepatotoxicity was proposed (13,14). The acute toxicity of PA was increased in enzyme-induced or GSH-depleted mice, but decreased in enzyme-inhibited mice or when the GSH level was maintained by cysteine pretreatment (13,14). Based on these results, a metabolic pathway by which PA could be detoxified by GSH was proposed. Pharmacokinetic data needed to support or negate this hypothesis, however, were not available.

TABLE I

Tissue Distribution of Radioactivity Derived from [^{14}C] Penicillic Acid in Mice after Intraperitoneal Administration (90 mg/kg∿0.5 μCi)

	Penicillic acid equivalent tissue concentration (μg/g)[a]			
	30 min	60 min	24 hr	48 hr
Urinary bladder	--- (11.2)	--- (21.7)	-- (0.1)	-- (0.1)
Gall bladder	--- (0.3)	--- (1.2)	-- (0.3)	-- (0.0)
Liver	110.9 (6.8)	61.6 (3.5)	14.3 (0.7)	5.4 (0.2)
Kidney	254.9 (5.2)	153.1 (3.2)	24.6 (0.4)	10.9 (0.2)
Heart	33.9 (0.2)	30.1 (0.2)	11.8 (0.1)	6.8 (0.0)
Lung	51.5 (0.3)	33.3 (0.2)	18.0 (0.1)	7.3 (0.0)
Testes	29.5 (0.3)	23.1 (0.2)	9.8 (0.1)	4.3 (0.0)
Spleen	82.6 (0.4)	53.2 (0.2)	13.9 (0.1)	5.6 (0.0)
Brain	5.3 (0.1)	4.8 (0.1)	1.5 (0.0)	1.1 (0.0)
Semivesicle	55.8 (0.4)	33.3 (0.2)	18.1 (0.1)	6.3 (0.0)
Fat	21.1 (0.3)	23.9 (0.2)	7.3 (0.2)	4.4 (0.1)
Muscle	16.2 (0.4)	23.8 (0.4)	5.1 (0.4)	3.0 (0.2)
Stomach	67.7 (1.8)	86.4 (1.9)	8.4 (0.2)	6.3 (0.1)
Duodenum	922.2 (18.8)	825.5 (11.1)	17.4 (2.2)	8.1 (0.1)
Jejunum	280.7 (8.4)	188.9 (4.5)	11.6 (0.5)	8.6 (0.4)
Ileum	266.5 (5.7)	224.7 (4.1)	23.1 (0.8)	10.4 (0.3)
Caecum	156.4 (2.8)	132.9 (2.7)	54.2 (1.1)	14.6 (0.3)
Large intestine	123.4 (3.3)	96.6 (2.2)	47.5 (1.1)	31.5 (1.2)
CO_2	---	---	-- (0.4)	-- (0.6)

[a]Each value represents the mean ± SE of at least 3 animals. Values in parentheses represent the % ± SE of dose recovered in tissues.

This paper reviews the pharmacokinetic data for PA in mice and its relationship to the active metabolite hypothesis.

EXPERIMENTAL PROCEDURES

Experimental procedures are briefly summarized; details have been published elsewhere (16,17).

The purity (>99%) of PA, produced as described by Chan et al. (14) was established by ultraviolet (UV) absorption, infrared, NMR, mass spectra, TLC and HPLC. The radiopurity (>99%) and chemical purity (>99%) of [^{14}C]-PA, a gift from Dr. A. Ciegler (USDA, New Orleans, LA), was confirmed by radiochromatography and HPLC, respectively. [^{14}C] Cysteine HCl was obtained from New England Nuclear (Cambridge, MA). All chemicals and organic solvents were of the highest purity available.

TABLE II

Tissue Distribution of Radioactivity Derived from [^{14}C] Penicillic Acid in Mice after Intravenous Administration (90 mg/kg∿0.5 μCi)

	Penicillic acid equivalent tissue concentration (μg/g)[a]	
	(1 hr)	(24 hr)
Urinary bladder	--- (9.8)	-- (0.1)
Gall bladder	--- (0.3)	-- (0.0)
Liver	32.5 (1.8)	6.1 (0.3)
Kidney	138.2 (1.8)	25.0 (0.4)
Heart	68.6 (0.5)	21.4 (0.1)
Lung	90.9 (0.7)	18.6 (0.2)
Testes	---	2.8 (0.0)
Spleen	33.8 (0.1)	13.4 (0.0)
Brain	12.3 (0.2)	3.3 (0.0)
Muscle	29.7 ---	11.2 ---
Stomach	17.4 (0.4)	5.0 (0.1)
Duodenum	395.5 (6.3)	12.6 (0.3)
Jejunum	92.9 (2.2)	5.0 (0.1)
Ileum	49.5 (1.2)	5.3 (0.1)
Caecum	30.0 (0.5)	8.5 (0.1)
Large intestine	24.6 (0.4)	9.7 (0.2)

[a]Each value represents the mean ± SE of at least 3 animals. Values in parentheses represent the % ± SE of dose recovered in tissues.

Animals and Treatments

Male ICR mice (33-36 g) from Charles River Mouse Farm (Wilmington, MA) were housed in artificially illuminated (12 hr/day) and temperature (72 ± 2 F)-controlled rooms free from known sources of toxic contaminants. Standard laboratory rodent chow (Ralston Purina Co., Richmond, IN) and water were freely available to animals at all times. PA and cysteine were dissolved in normal saline. Diethylmaleate (DEM) was dissolved in corn oil. PA was administered either orally, ip or iv. [^{14}C] PA (0.5 Ci) was administered via the tail vein. Bile was collected by cannulation of the common bile duct with PE-10 polyethylene tubing after the gall bladder was ligated.

Radioactivity Assay

Tissue, fecal matter and blood samples were oxidized in a Packard Model 306-Tri-Carb Sample Oxidizer. The CO_2 was trapped with 8 mL of Carbo-Sorb and counted for radioactivity after adding 10 mL of Permafluor V. Plasma, urine and bile samples were counted for radioactivity after adding 10 mL of PCS scintillation fluor.

Extraction

Extraction procedures for authentic PA from plasma, urine and bile were as described by Chan et al. (16). For PA and its metabolites in urine and bile, the following procedures were adopted. Three volumes of 0.2 M acetate buffer solution (buffer pH = 5.0 for urine, and pH = 4.4 for bile) were added to urine or bile samples. The mixture was extracted twice with 3 volumes of ethyl acetate by mixing each extraction for 2 min and centrifugated at 600 × g for 10 min with a table-top centrifuge. The organic phase was separated and evaporated under N_2. The remaining aqueous phase was centrifuged again and the clear layer was transferred into a test tube and evaporated to dryness under N_2.

High Pressure Liquid Chromatography (HPLC)

Reversed-phase chromatography was performed using a Waters Associates HPLC system (Milford, MA) with a precolumn, a guard column and a C-18 μBondapak analytical column (18). The elution systems were optimized based on sensitivity and separation of PA or its metabolite(s)

from interfering substances. Two systems containing distilled-in-glass acetonitrile (AN) or methanol (MeOH) (Burdick and Jackson Labs, Muskegon, MI), glass distilled water (H_2O) and 0.5% glacial acetic acid (Ac) (Fisher Scientific Co., Fairlawn, NJ) were used. For the determination of plasma authentic PA, the mobile phase consisted of AN, H_2O and 0.5% Ac in a volume ratio of 25:75:0.3. For the separation of urinary and biliary PA metabolites, the mobile phase consisted of MeOH, H_2O, 0.5% Ac in a volume ratio of 13:87:0.3. The flow rate was 1.2 mL/min in both systems. The HPLC column eluates, collected at 10-sec intervals, were dissolved in 10 mL PCS fluor for liquid scintillation spectrometry. Radioactivity profiles of the column eluates were corrected with the estimated lag time.

Glucuronide and Sulfate Conjugate

Urine samples were subjected to analysis for glucuronide and sulfate conjugates according to the methods of Talalay et al. (19) (Sigma Chemical Co., St. Louis, MO) and Dodgson and Spencer (20), respectively.

Kinetics of PA

An aliquot (100-200 μL) of each plasma sample collected after oral, iv, and ip administration of PA (90 mg/kg) was extracted and analyzed for PA by HPLC (18). The plasma PA concentration (Cp) was fitted to a 1-, 2- or 3-compartment open model using the Non-Linear-Least-Squares Regression Analysis (NLIN) computer program in the Statistical Analysis System (SAS User's Guide, 1979). The iterative algorithm used was that proposed by Marquardt (21).

Student's unpaired t-test was used for statistical analysis and $p < 0.05$ was chosen as significant level (22).

RESULTS

Oral Absorption

Absorption of PA after gastric gavage was fast with an absorption rate constant estimated to be 0.0698 min^{-1} (data not shown).

Tissue Distribution

Tissue distribution of radioactivity after ip and iv administration of [^{14}C] PA is shown in Tables I and II, respectively. In general, organs such as liver, kidneys, heart, lungs and spleen contained higher levels of radioactivity than tissues such as brain, muscle and fat. While liver and kidneys contained the highest concentration of tissue radioactivity and the highest percentage of dose after either ip and iv administration, the percentage of dose recovered in the liver at 1 and 24 hr was significantly higher after ip than after iv administration of [^{14}C] PA. However, a significantly higher concentration of radioactivity in the lungs and in the heart was observed after iv than after ip administration. The concentration of radioactivity in the kidneys after both ip and iv administration of [^{14}C] PA was similar.

After ip administration of [^{14}C] PA, ca. 0.4, 0.44 and 10% of the dose were recovered in the urinary bladder, gallbladder and duodenum, respectively, as early as 10 min (data not shown). By 30 min, 40% of the dose was recovered in the GI tract and 11% in the urinary bladder (Table I). Over 45% of the dose was recovered in the urinary bladder in 2 hr (data not shown). A higher concentration of radioactivity was recovered from the small intestine, particularly in the duodenum, than from other portion of the GI tract (Tables I and II).

The subcellular distribution of radioactivity in the kidneys and liver after administration of [^{14}C] PA is shown in Table III. Most of the recovered radioactivity (85%) was in the cytosol fraction.

TABLE III

Subcellular Distribution of Radioactivity after ip Administration of [^{14}C] Penicillic Acid in the Liver and Kidney

Fraction[a]	Recovered radioactivity (%)[b]	
	Liver	Kidney
Nuclear and debris	8.12 ± 0.7	8.23 ± 0.81
Mitochondrial	1.08 ± 0.6	2.08 ± 0.94
Microsomal	6.31 ± 0.21	3.35 ± 0.95
Cytosol	84.5 ± 0.83	85.6 ± 1.98

[a]Animals were sacrificed 20 min after the administration of penicillic acid (90 mg/kg, ip) containing 1 μCi of [^{14}C] penicillic acid. Fractions were prepared by differential centrifugation.

[b]Each value represents the $\overline{X}$ ± SE of at least 3 animals. Recovery of radioactivity after differential centrifugation was 68.9%.

Excretion

Ca. 2.5 and 0.35% of the administered dose were excreted in the feces and CO_2, respectively, in 24 hr after ip administration of [^{14}C] PA (90 mg/kg) (Table I). Ca. 12% of the iv-administered dose was excreted in the bile by 10 min and 25% by 1 hr (Fig. 1). The biliary excretion of [^{14}C] PA was decreased by DEM pretreatment. Radioactivity was rapidly excreted in the urine after administration of [^{14}C] PA. Over 60% of an ip dose and ca. 90% of an iv dose (90 mg/kg) were excreted in the urine (data not shown).

Kinetic Analysis

Kinetic analysis of plasma PA concentration (data not shown) showed that different pharmacokinetic models were

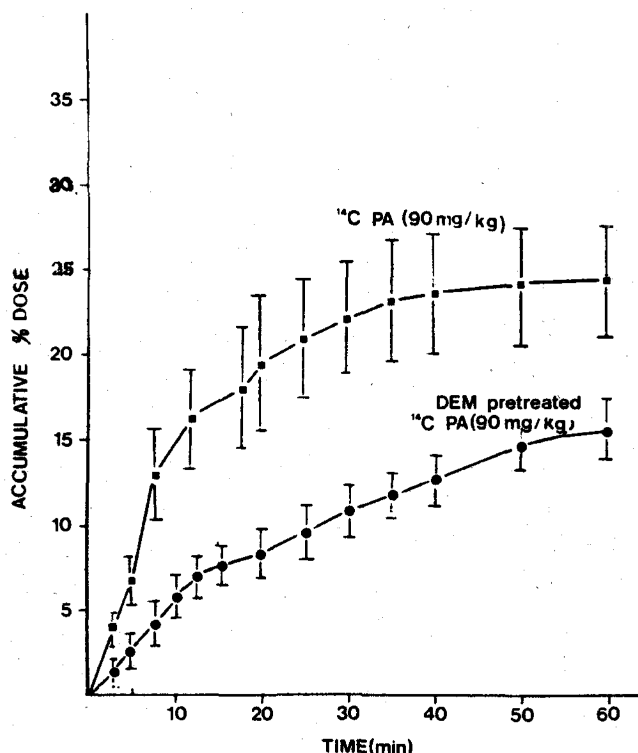

FIG. 1. Radioactivity excreted in the bile of mice treated with penicillic acid (PA) (90 mg/kg, iv): (■); and mice pretreated with diethylmaleate (DEM) and then treated with PA (90 mg/kg, iv): (●). Ca. 0.5 μCi of [^{14}C] PA was administered to each animal. Each point represents $\overline{X}$ ± SE of 3 mice.

TABLE IV

Recovery of Total Radioactivity in Different Extraction Systems from Urine and Bile of Mice Treated with [^{14}C] Penicillic Acid (PA)

Extraction systems	Solvent phase	Recovery (%)[a] Urine	Bile
I. pH = 5.0	Aqueous	90.30 ± 0.64	99.10 ± 0.09
	Ethylacetate	9.70 ± 0.24	0.91 ± 0.03
II. pH = 5.0	Aqueous	98.00 ± 0.42	ND[b]
	$CHCl_3$	1.97 ± 0.55	ND
III. pH = 8.0	Aqueous	99.80 ± 0.16	ND
	$CHCl_3$	0.91 ± 0.01	ND
IV. pH = 5.0	Aqueous	93.50 ± 0.76	ND
	Ether/ethanol (3:1)	4.37 ± 0.37	ND
	$CHCl_3$	2.30 ± 0.85	ND

[a]Each value represents the $\bar{X}$ ± SE of at least 3 animals. Urine was collected for 24 hr from mice treated with PA (90 mg/kg, ip ∿ 0.5 µCi [^{14}C] PA), and bile was collected for 1 hr from mice treated with PA (90 mg/kg, iv, 0.5 µCi [^{14}C] PA).

[b]ND = None detected.

needed to fit the data after oral, ip, or iv administration of PA. One-, 2- and 3-compartment models better described the plasma concentration vs time after oral, ip, and iv administration of PA according to the following equations:

$$c_p = B\,(e^{-\beta t} - e^{-kat}) \quad [I]$$

$$c_p = Ae^{-\alpha t} + Be^{-\beta t} - Ce^{-kat} \quad [II]$$

$$c_p = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}, \quad [III]$$

where c_p = plasma PA concentration, ka = first order absorption rate constant, β = overall constant, α, π = hybrid rate constant, A, B, C, P = concentration constants.

Metabolism

The recovery of total radioactivity by the different extraction systems from urine and bile from mice treated with [^{14}C] PA are listed in Table IV. Over 90% of the PA metab-

TABLE V

Glucuronides Derived from [^{14}C] Penicillic Acid (PA) in the Urine of Mice Treated with PA

Time[b] (hr)	Glucuronides[a] (% of urine total radioactivity)
8	16.1 ± 3.0
16	7.8 ± 6.5
24	10.23 ± 3.53
48	0
72	0
96	0

[a]Each value represents the $\bar{X}$ ± SE of at least 3 animals.

[b]Time after the administration of PA (90 mg/kg, ip) containing ca. 0.5 µCi of [^{14}C] PA.

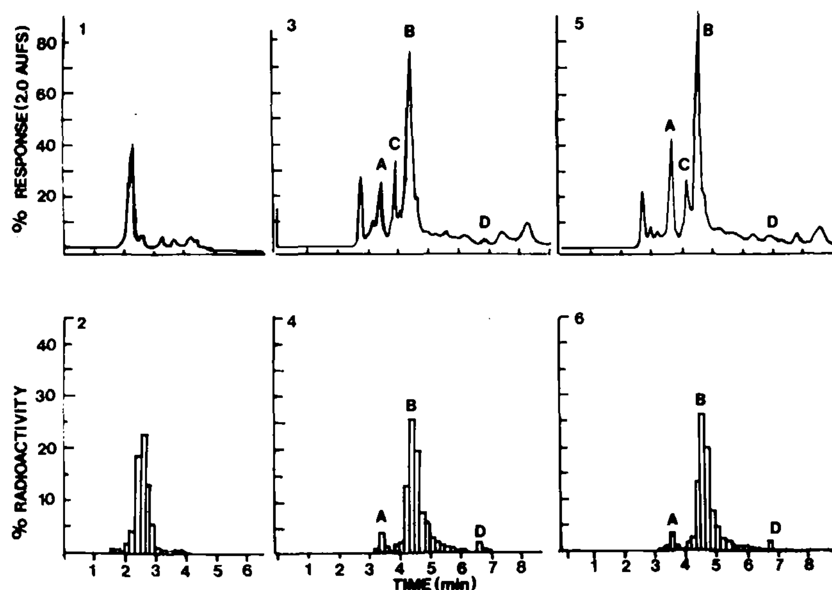

FIG. 2. HPLC chromatogram tracings and radioactivity profiles of column eluates of the aqueous extracts of bile from mice treated with [^{14}C] penicillic acid (PA) using HPLC systems containing methanol, water and 0.5% acetic acid (Ac). The aqueous extracts were obtained after the bile was extracted with ethyl acetate. Panel 1, 2: from mice treated with [^{14}C] cysteine only; panels 3, 4: from mice treated with [^{14}C] cysteine and then with unlabeled PA (90 mg/kg, ip); panels 5, 6: from mice treated with [^{14}C] PA (90 mg/kg, iv∿0.5 µCi). HPLC system: Methanol/H_2O/Ac (0.5%) = 13.87:0.3, flow rate = 1.2 mL/min. The column eluate was collected at 10-sec intervals.

olite(s) in urine and 99% in bile could not be extracted with polar organic solvents.

No sulfate conjugates of PA were detected in the urine. However, 16, 7 and 10% of the metabolites detected in the urine at 8, 16 and 24 hr after administration of PA (90 mg/kg, ip) were glucuronide conjugates, respectively (Table V).

The HPLC chromatographic tracings and radioactivity profiles of column eluate of aqueous extracts of bile and urine from mice treated with [^{14}C] PA or [^{14}C] cysteine are shown in Figures 2 and 3, respectively. The retention of PA in this system was 830 sec. At least 3 PA metabolites derived from cysteine or GSH were found in the aqueous extract of bile (obtained after the bile was extracted with ethyl acetate) (Fig. 2). Radioactivity peaks (A, B and D), corresponding to metabolites in the bile from mice treated with [^{14}C] cysteine and then with unlabeled PA, had identical retention times as radioactivity peaks corresponding to metabolites in the bile from mice treated only with [^{14}C]-PA (Fig. 2, panels 4 and 6). These radioactivity peaks, however, are different from radioactivity peaks from mice treated only with [^{14}C] cysteine (Fig. 2, panel 2), indicating that the radioactivity peaks shown in panel 2 were unrelated to PA. These metabolites also could be identified in the HPLC chromatogram tracings (Fig. 2, panels 3 and 5). At least 2 metabolites (peaks B′ and D′), nonextractable with ethyl acetate, were noted in the HPLC chromatogram tracings and at least 3 radioactivity peaks were detected in the aqueous extract of urine from mice treated with [^{14}C] cysteine and then with unlabeled PA (Fig. 2, panels 3 and 4). The chromatogram tracing and radioactivity peaks were identical to metabolic profiles of urine collected from mice treated only with [^{14}C] PA (Fig. 3, panels 5 and 6), but different from those peaks from mice treated only with [^{14}C] cysteine (Fig. 3, panels 1 and 2), indicating that the radioactivity peaks shown in panel 2 were unrelated to PA.

DISCUSSION

The different kinetic patterns of PA after different routes of administration are important in the understanding of its mechanism of toxicity. PA was most toxic by ip administration (90 mg/kg), followed by iv (250 mg/kg) and oral routes (600 mg/kg) of administration (13,23). If toxic metabolites are produced in the liver, the hepatic first pass effect after ip administration may be attributed to the ip route being the most toxic. Dilution of PA in an additional compartment besides the liver may explain the reduced toxicity following iv administration. The one compartment model for the oral route may be the result of an artifact of slow adsorption from the GI tract and/or interaction between PA and intestinal contents, thus explaining the least toxicity of PA by this route.

Hepatic metabolism of PA was supported by the fact that more than 25% of an administered dose was excreted in the bile in1 hr and most of the radioactivity in the bile was in the form of polar metabolites. The depression of biliary excretion of PA in DEM-pretreated animals suggests that GSH may be a limiting factor in the metabolism of PA.

Urinary excretion of PA was rapid with only a trace of the radioactivity in the urine being authentic PA. Most of the urinary radioactivity could be attributed to polar metabolites. Separation of these metabolites in the aqueous extracts of bile and urine was extremely difficult. However, at least 3 PA metabolites in bile and 3 metabolites in the urine could be separated. These metabolite(s) were GSH or cysteine derivatives. A possible candidate for a urinary metabolite is the N-acetyl derivatives of the cysteine conjugates occurring in the bile. Our earlier proposed 2-GSH-dependent detoxification pathway (14) was substantiated by the isolation of different GSH or cysteine derivatives. These GSH-dependent pathways were further supported by the fact that most of the radioactivity in the liver was located in the cytosol fraction where GSH is found in greatest quantity. A glucuronide conjugate of PA also was a major urinary metabolite.

Based on these data, a metabolic pathway for PA was proposed (Fig. 4) which includes hepatic bioactivation,

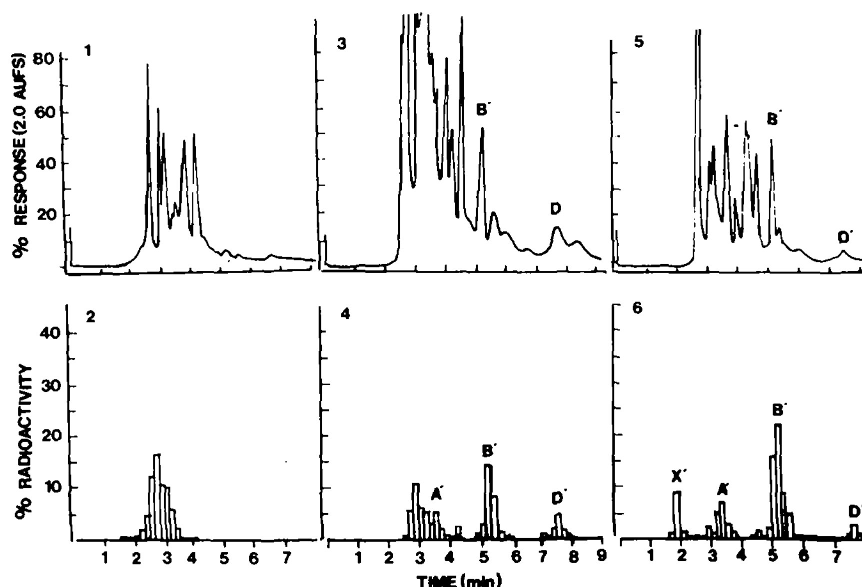

FIG. 3. HPLC chromatogram tracings and radioactivity profiles of column eluates of the aqueous extracts of urine from mice treated with [^{14}C] penicillic acid (PA) using HPLC system containing methanol, water and 0.5% acetic acid (Ac). The aqueous extracts were obtained after the urine was extracted with ethyl acetate. Panel 1, 2: from mice treated with [^{14}C] cysteine only; panels 3, 4: from mice treated with [^{14}C] cysteine and then with PA (90 mg/kg, ip). Panels 5, 6: from mice treated with [^{14}C] PA (90 mg/kg, iv∿0.5 μCi). HPLC system: Methanol/H_2O/Ac (0.5%) = 13.87:0.3, flow rate = 1.2 mL/min. The column eluate was collected at 10-sec intervals.

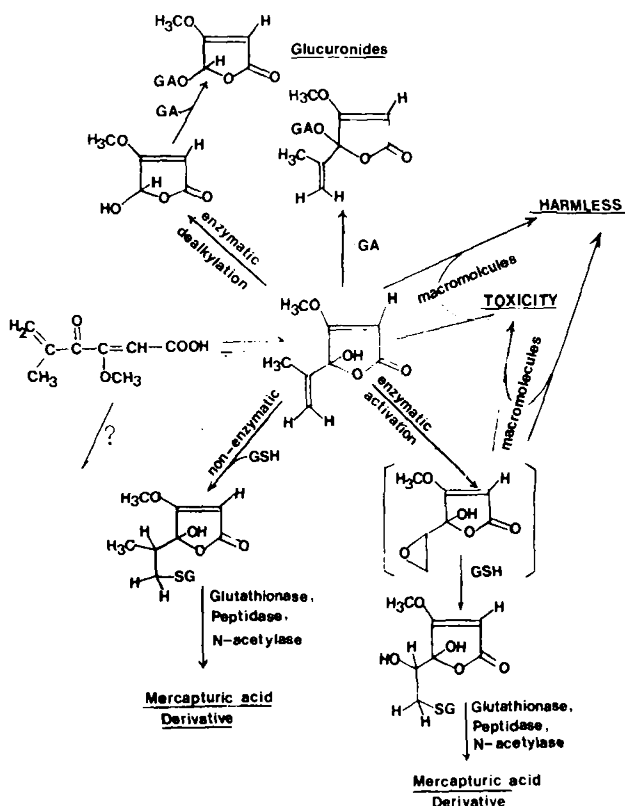

FIG. 4. Proposed metabolic pathways of penicillic acid. GSH = reduced glutathione; GA = glucuronic acid.

GSH conjugation (both nonenzymatically or via the enzymatic bioactivation), formation of mercapturic acid and glucuronidation. GSH is the major limiting factor in the toxicity, metabolism and excretion of PA.

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REFERENCES

1. Scott, P.M., Mycotoxic Fungi, Mycotoxins and Mycotoxicoses, "An Encyclopedia Handbook," Vol. 1, edited by D. Wyllie and L.G. Morehouse, Marcel Dekker, Inc., New York and Basel, 1978, p. 311.
2. Dickens, F., and H.E.H. Jones, Br. J. Cancer 15:85 (1961).
3. Dickens, F., and H.E.H. Jones, Ibid. 17:100 (1963).
4. Dickens, F., and H.E.H. Jones, Ibid. 19:392 (1965).
5. Kuntzman, C.P., and A. Ciegler, Appl. Microbiol. 20:204 (1970).
6. Pero, R.W., D. Harven, R.G. Owens and J.P. Snow, J. Chromatogr. 65:501 (1973).
7. Bacon, C.W., J.G. Sweeney, J.D. Robbins and C. Burdick, Appl. Microbiol. 26:155 (1973).
8. Thorpe, C.W., and R.L. Johnson, J. Assoc. Offic. Anal. Chem. 57:861 (1974).
9. Snow, J.P., G.B. Lucas, R.W. Harven and R.G. Owens, Appl. Microbiol. 24:34 (1972).
10. Ciegler, A., H.J. Mintzlaff, D. Weisleder and L. Leistner, Ibid. 24:114 (1972).
11. Hayes, A.W., P.D. Unger and W.L. Williams, Ann. Nutr. Aliment. 31:711 (1977).
12. Umeda, M., Jpn. J. Exp. Med. 41:195 (1971).
13. Chan, P.K., C.S. Reddy and A.W. Hayes, Toxicol. Appl. Pharmacol. 52:1 (1979).
14. Chan, P.K., A.W. Hayes, E.F. Meydrech and A. Ciegler, Ibid. 55:291 (1980).
15. Chan, P.K., and A.W. Hayes, J. Toxicol. Environ. Health 7:169 (1981).
16. Chan, P.K., A.W. Hayes, M.J. Siraj and G.F. Meydrech, Drug Disposition and Metabolism (submitted).
17. Chan, P.K., A.W. Hayes and M.J. Siraj, Ibid. (submitted).
18. Chan, P.K., M.J. Siraj and A.W. Hayes, J. Chromatogr. 194: 387 (1980).
19. Talalay, P.W., W.H. Fishman and C. Huggins, J. Biol. Chem. 166:757 (1946).
20. Dodgson, K.S., and B. Spencer, Biochem. J. 55:315 (1953).
21. Marquardt, D.W., J. Soc. Ind. Appl. Math 11:431 (1963).
22. Steel, R.G.D., and J.H. Torrie, "Principles and Procedures of Statistics, McGraw-Hill," New York, 1960.
23. Murnaghan, M.F., J. Pharmacol. Exp. Ther. 88:119 (1946).