Quinoline: Conversion to a Mutagen by Human and Rodent Liver[1,2]

Monica Hollstein,[3] Ronald Talcott,[3] *and* Eddie Wei[3]

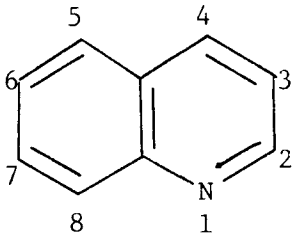
ABSTRACT—Quinoline, a hepatocarcinogen in rats, and 23 quinoline derivatives were tested for mutagenic activity with the Ames *Salmonella typhimurium* assay. Quinoline, 5-hydroxyquinoline, and 8-hydroxyquinoline were mutagenic in strain TA 100 when Aroclor 1254-induced rat (male outbred Sprague-Dawley) liver homogenate was present in the incubation mixture. Enzyme preparations from rats pretreated with P-448-dependent aryl hydrocarbon hydroxylase inducers [3-methylcholanthrene (MCA) and β-naphthoflavone] and MCA-treated "responsive" C57BL mice also metabolized quinoline to a mutagen, but phenobarbital and pregnenolone-16α-carbonitrile pretreatment did not yield active preparations. The mutagenicity of quinoline was blocked by the in vitro addition of menadione, butylated hydroxytoluene, α-naphthoflavone, vitamin A acetate, and glutathione to the test system. Depletion of glutathione by diethyl maleate pretreatment in vivo enhanced the mutagenic potential of the liver enzyme preparation. Mutagenic activity was correlated to the formation of water-soluble quinoline metabolites, and we suggested that the reactive quinoline intermediate is quinoline-2,3-epoxide. Microsomal enzymes isolated from human liver tissue, but not lung tissue, also converted quinoline to a mutagen.—J Natl Cancer Inst 60: 405–410, 1978.

Quinoline is enzymatically converted to a mutagen in the Ames bacterial assay (*1*). When fed to rats, it produces benign and malignant tumors of the liver (*2*). Little is known about the experimental conditions or metabolic pathways by which quinoline is converted to a mutagen or a carcinogen. In this investigation, we examined: 1) a number of quinoline derivatives for mutagenic activity, to determine some of the structural requirements of the quinoline nucleus necessary for mutagenesis; 2) the effects of some inducers of liver mixed-function oxidases on the activity of enzymes that metabolize quinoline to a mutagen; 3) compounds that are in vitro inhibitors of quinoline mutagenesis; and 4) the metabolites of quinoline that are associated with mutagenesis. The ability of human liver and lung to activate quinoline was also examined because quinoline and its derivatives are widely used in the chemical industry and in drugs and are formed in the body from tryptophan metabolism. A preliminary account of these results has been published (*1*).

MATERIALS AND METHODS

Chemicals and bacteria.—Commercially available biochemicals were obtained from Sigma Chemical Co., St. Louis, Missouri; Aldrich Chemical Co., Milwaukee, Wisconsin; Calbiochem, La Jolla, California; and ICN-K&K Laboratories Inc., Plainview, New York. 8-Hydroxyquinaldic acid was a gift from Dr. D. E. Mullins (Virginia Polytechnic Institute, Blacksburg, Va.). 3-Hydroxyquinoline was synthesized in our laboratory according to the method of Icke (*3*). Radioactive quinoline was prepared by the Tritium Labelling Service, Amersham/Searle Corp., Chicago, Illinois, refined to 95% radiochemical purity on glass silica gel thin-layer chromatography plates, and redissolved in methanol. The specific activity of the purified preparations was 450 mCi/mmole. Strains TA 98, 100, 1535, and 1537 of *Salmonella typhimurium* were provided by Dr. Bruce Ames, University of California, Berkeley.

S. typhimurium *mutagen assay.*—This bioassay is based on the ability of chemicals to revert mutant strains of *S. typhimurium* from a histidine requirement back to prototrophy (*4*). The pour plate procedure, detailed by Ames et al. (*5*), was used without modification for testing quinoline and its derivatives. Quinoline (text-fig. 1) and its derivatives were assayed in the four



TEXT-FIGURE 1.—Quinoline.

standard tester strains (TA 98, 100, 1535, and 1537) at three dose levels (10 μg, 100 μg, and 1 mg per plate) with and without ARO-induced rat liver enzymes and the appropriate cofactors. A threefold or greater increase in the spontaneous mutation frequency on plates showing no signs of toxicity was used as the criterion for a positive mutagenic response (*5*).

Preparation of tissue homogenates.—Tissue samples of liver, lung, and small intestine were obtained from male Sprague-Dawley outbred rats (190–210 g) and male

ABBREVIATIONS USED: ARO = Aroclor 1254; MCA = 3-methylcholanthrene; PCN = pregnenolone-16α-carbonitrile; β-NF = β-naphthoflavone; PB = phenobarbital sodium; DEM = diethyl maleate; BHT = butylated hydroxytoluene; α-NF = α-naphthoflavone; AHH = aryl hydrocarbon hydroxylase.

[1] Received June 14, 1977; accepted August 10, 1977.

[2] Supported by training grant T01-OH00020 from the National Institute of Occupational Safety and Health and by the University of California Cancer Research Fund.

[3] Department of Environmental Health Sciences, School of Public Health, University of California, Berkeley, Calif. 94720.

C57BL and DBA mice (20–24 g) maintained on Purina Laboratory Chow and water ad libitum. Animals were killed by cervical dislocation, and their livers and lungs were rapidly excised and rinsed in ice-cold 0.15 M KCl. Epithelial tissue from the lumen of the small intestine was obtained by scraping the intestinal surface with a glass cover slip. The collected tissues were then separately homogenized in two volumes of sterile cold KCl and centrifuged at 9,000×*g* for 10 minutes; the supernatant fraction (S-9) was stored at −70° C in 3-ml portions. A sample of human liver and lung, obtained from a 20-year-old male accident victim (provided by Dr. Susan Rice, Anesthesiology Research, Stanford Veterans Administration Hospital, Palo Alto, Calif.) and frozen at −70° C for 2 days after autopsy, was thawed in sodium phosphate buffer (pH 7.4), minced, homogenized, centrifuged, and prepared for storage as described above. The tissues were kept chilled on ice throughout the 45- to 60-minute preparation period.

The protein content of the S-9 fractions was measured by the biuret method (*6*). For each assay, the amount of S-9 fraction added to the plate was adjusted so that an equal amount of protein was present, regardless of the source of S-9. All S-9 preparations from lung and intestine were sterilized by filtration through a 0.45-μ Millipore filter before use. Lung homogenates of protein concentrations greater than 3 mg/plate were not tested because such concentrations could not be filtered efficiently.

Oxidative enzymes in tissues are induced after pretreatment with certain chemicals. To determine the effect of inducers on the enzymes that convert quinoline to a mutagen, the following chemicals were given under standardized dosage conditions: 1) ARO was dissolved in corn oil and administered ip to rats in a single injection at 500 mg/kg (*5*). Tissues were obtained on day 5 after injection. 2) MCA or quinoline was dissolved in corn oil and administered to rats in three daily ip injections at 80 mg/kg (*5*) and 20 mg/kg, respectively. Tissue homogenates were prepared 24 hours after the last injection. Pretreatment of mice consisted of a single ip dose (80 mg/kg) of MCA 48 hours before they were killed (*7*); controls received corn oil alone. 3) PCN or β-NF was suspended in 0.9% sterile NaCl and 2 drops of Tween 80 and administered to rats at 50 mg/kg (PCN) or 80 mg/kg (β-NF) in 3 daily ip injections (*8*). Tissue homogenates were prepared 24 hours after the last injection. 4) PB (0.1%) was dissolved in the drinking water of rats 1 week before they were killed (*4*). 5) DEM depletion of liver glutathione in ARO-treated rats was accomplished by giving the animals injections of 0.3 ml DEM/kg one-half hour before they were killed (*9*).

Liver preparations, containing the pooled tissues from at least 2 pretreated aimals, were tested in duplicate at two concentrations of quinoline per plate (100 and 500 μg). The ability of different tissue preparations to metabolize quinoline to a mutagen was assessed by an activity index calculated by division of the number of TA 100 revertants on plates containing quinoline and tissue homogenate (at optimum protein concentrations) by the number of TA 100 revertants on plates containing quinoline alone.

In vitro inhibition of quinoline mutagenicity.—Several compounds that inhibit the oxidative metabolism of xenobiotics by liver enzymes were tested in the Ames mutagenesis assay to determine their effect on quinoline mutagenicity. The chemicals were dissolved in dimethyl sulfoxide (vitamin A acetate, BHT, α-NF, methylmercaptoimidazole, and menadione) or sterile water (glutathione and cysteine), and 0.1 ml of the solution was added to the top agar containing 500 μg of quinoline and the ARO-induced S-9 mix. The assay procedure was followed as described above without further modification. The chemicals were tested at 5, 12.5, 25, and 50 μmoles per plate and were also screened for mutagenicity and toxicity at these concentrations on plates containing microsomal mix but no quinoline. None was mutagenic to TA 100, and no toxicity to the tester strain was evident at the concentrations listed in table 2 (*see* "Results").

In vitro metabolism of [^{3}H]quinoline by liver microsomes.—Rat liver homogenates convert quinoline to a mutagen only after certain pretreatment conditions. The pattern of quinoline metabolites associated with mutagenicity was therefore investigated by incubation of [^{3}H]quinoline with different preparations of liver microsomal enzymes. Quinoline metabolites were initially categorized as nonpolar (soluble in chloroform–isoamyl alcohol, 17:3) or water-soluble, and the pattern of metabolites was determined for optimal reaction conditions and different pretreatment schedules.

Incubation flasks contained 5 μCi of [^{3}H]quinoline plus sufficient nonradioactive quinoline to yield concentrations of 10 μM, 100 μM, 1 mM, 5 mM, or 10 mM in 0.1 ml dimethyl sulfoxide:methanol (1:1); 1 ml cofactor mix, described in the Ames assay procedure above; 2 U glucose-6-phosphate dehydrogenase; 5 mg microsomal protein; and 0.2 M sodium phosphate buffer (pH 7.4) to a total volume of 3 ml. The microsomal protein was prepared by centrifugation of the 9,000×*g* supernatant fraction of ARO- or corn oil-treated liver homogenate in 0.15 M KCl and 8 mM $CaCl_2$ at 15,000×*g* for 15 minutes. Control incubation tubes contained 5 μCi of quinoline (5 mM), microsomal protein, and all cofactors except $NADP^+$. The reaction was allowed to proceed for 25 minutes at 37° C in a metabolic shaker. Addition of 1.6 ml chloroform–isoamyl alcohol (17:3) stopped the reaction. The tubes were spun in vortex for 30 seconds and centrifuged at 5,000×*g* for 5 minutes; the protein layer was discarded. A 0.1-ml portion of the organic phase (containing unreacted quinoline and nonpolar metabolites) and 0.1 ml of the aqueous phase were added to scintillation cocktail (700 ml toluene, 300 ml ethanol, 8 g 2,5-diphenyloxazole, and 0.5 g 1,4-bis[2-(4-methyl-5-phenyl-oxazolyl)]benzene/liter). Radioactivity was measured for 1 minute in a Packard Model 3330 scintillation counter.

Total recovered radioactivity was calculated by adding the total number of counts in 1.6 ml organic extract to the total number of counts remaining in 3 ml aqueous incubation mixture after extraction. To determine the

percent of radioactivity in the organic phase contributed by unmetabolized quinoline, 40 μl of the chloroform–isoamyl alcohol extract was spotted on silica gel plates (Merck silica gel 60) and developed in a closed chamber containing chloroform:acetic acid:methanol:water (100:2:2:5). UV fluorescence of a cold quinoline standard chromatogram and a chromatogram of 10 μl [^{3}H]quinoline stock solution located the quinoline-containing sections of the experimental chromatograms. The quinoline bands were cut from the experimental chromatograms and counted in scintillation fluid. The remainder of each chromatogram, containing nonpolar metabolites of quinoline, was also counted. Radioactivity counts in the aqueous phase, the quinoline band, and the remaining bands of each silica gel plate were multiplied by the appropriate volume factors and divided by the total recovered counts to obtain the percent of total recovered radioactivity contributed by polar metabolites, unreacted quinoline, and nonpolar metabolites, respectively.

Microsomal enzymes from liver S-9 induced with specific oxidative enzymes and tested in the Ames microsomal assay for their ability to metabolize quinoline to a mutagen were assayed in a series of incubations. The S-9 fractions from rats treated with MCA, quinoline, PCN, PB, β-NF, ARO and ARO plus DEM were used to prepare microsomes for these in vitro metabolism studies. The same incubation conditions were followed with minor alterations: Microsomal metabolism at only one concentration of quinoline was tested (100 μM), and 2 mg protein/ml was added to each incubation mixture. Extraction and counting procedures are as described above. Total recovered radioactivity and the percent of recovered radioactivity contributed by polar metabolites, unreacted quinoline, and nonpolar metabolites were calculated for each incubation mixture.

RESULTS

Mutagenic Activities of Quinoline Compounds

The mutagenic activities of quinoline and its derivatives are shown in table 1. In the first group of compounds (industrial chemicals), quinoline and 5-hydroxyquinoline were mutagenic with a specific activity approaching that reported for benzidine (*10*). Other compounds in this group were either nonmutagenic or were marginally positive at the highest dose tested (1 mg/plate): 4(*H*)-quinoline, quinoxaline, 2,4-quinoline-

TABLE 1.—*Mutagenicity in the Ames bioassay of quinoline and 23 derivatives*

Compound (dose[a]/plate)	Revertants[b]								Source
	TA 98		TA 100		TA 1535		TA 1537		
	−S-9	+S-9	−S-9	+S-9	−S-9	+S-9	−S-9	+S-9	
Industrial chemicals:									
Quinoline (100 μg)	18	59	157	*960 (6,000)*[c]	5	23	0	0	Pfaltz and Bauer[d]
2-Hydroxyquinoline (100 μg)	10	48	114	128	12	11	5	12	ICN-K&K
3-Hydroxyquinoline (100 μg)	26	18	82	70	10	10	3	9	Synthesized (*3*)
4-Hydroxyquinoline (100 μg)	9	17	113	135	25	21	2	7	Aldrich
5-Hydroxyquinoline (100 μg)	8	**49**	111	*439*	8	14	8	17	Pfaltz and Bauer[d]
6-Hydroxyquinoline (100 μg)	13	30	115	167	13	23	23	12	ICN-K&K
7-Hydroxyquinoline (100 μg)	18	32	130	135	10	6	10	9	"
Quinoline-*N*-oxide (1 mg)	27	47	80	171	13	12	7	6	"
8-Hydroxyquinoline-*N*-oxide (1 mg)	17	30	129	174	17	21	21	**32**	Aldrich
Isoquinoline (100 μg)	17	29	78	100	9	13	7	12	"
Isocarbostyril (1 mg)	22	9	102	94	7	15	2	5	"
5-Hydroxyisoquinoline (100 μg)	24	12	64	109	12	20	13	26	"
2,4-Quinolinediol (1 mg)	35	**101**	100	123	9	10	5	17	"
4(*H*)-Quinoline (1 mg)	17	54	122	**249**	14	*96*	6	19	ICN-K&K
8-Hydroxyquinaldine (100 μg)	16[e]	12[e]	75	100	13[e]	6[e]	1	8	Aldrich
Quinoxaline (1 mg)	17	*138*	85	87	8	17	9	14	"
3-Quinoline carbonitrile (1 mg)	11	27	135	203	7	15	5	10	"
Endogenous compounds:									
Quinaldic acid (1 mg)	10	31	118	119	8	11	4	2	Pfaltz and Bauer[d]
8-Hydroxyquinaldic acid (1 mg)	14	36	125	193	9	11	9	5	Dr. Mullins
Xanthurenic acid (1 mg)	22	13	117	134	14	27	13	23	Sigma
Food and drug products:									
Vioform (1 μg)	21	16	129	117	13	10	0	10	Aldrich
8-Quinolinesulfonic acid (1 mg)	20	18	166	163	7	12	7	6	"
8-Hydroxyquinoline (100 μg)	20	78	68	*685*[f]	0	0	0	0	ICN-K&K
Quinoline yellow (1 mg)	19	22	100	141	7	6	3	6	"
Dimethyl sulfoxide (solvent control)	24	36	110	121	12	24	8	12	

[a] Dose at which the highest mutagenic activity was observed, or the highest nontoxic dose tested.
[b] *Numbers in italics* indicate positive results; *numbers in boldface* indicate marginal values.
[c] *Number in parentheses*: number of TA 100 revertants at a test dose of 1 mg.
[d] Flushing, N. Y.
[e] Number of revertants at a test dose of 10 μg; higher concentrations toxic to this strain.
[f] Number of revertants at a test dose of 40 μg; higher doses yielded a decrease in mutagenic activity (*1*).

diol, 5-hydroxyisoquinoline, and 8-hydroxyquinoline-*N*-oxide were weakly positive in various tester strains. The mutagenicity of the parent compound quinoline was expressed only in strain TA 100. None of the tryptophan metabolites were positive in any of the tester strains, a result in accord with the observations of Bowden et al. (*11*). Of the drugs and food additives tested, only 8-hydroxyquinoline was mutagenic; at doses above 100 μg/plate it became toxic to the tester strains (*1*). Iodochlorhydroxyquin (Vioform) was toxic to the bacterial tester strains at doses as low as 10 μg/plate. Its mutagenic activity could not, therefore, be ascertained in this bioassay. Quinoline yellow and 8-quinoline sulfonic acid were not mutagenic.

Enzymatic Requirements for Quinoline Mutagenicity

Liver mixed-function oxidases can be induced by more than 200 chemicals. The specific characteristics of the induced enzymes can be used for broad categorization into two groups (*12*): group 1 is exemplified by MCA, and group 2 is exemplified by PB. MCA and β-NF selectively induce cytochrome P-448-dependent AHH, but PB and PCN induce a different spectrum of P-450 dependent mixed function oxidases (*8*). ARO pretreatment stimulates the activities of P-448-dependent (MCA-inducible) and P-450-dependent (PB-inducible) enzymes (*13*). ARO is, therefore, the inducer of choice for the preparation of the S-9 fractions used in the Ames test (*4*). Quinoline was mutagenic when liver enzymes were obtained from rats pretreated with either of two chemicals from group 1 (MCA and β-NF) but not with two chemicals from group 2 (PB and PCN) (table 2). MCA induced its characteristic enzymes in C57BL but not in DBA mice. Liver enzymes prepared from MCA-pretreated C57BL mice activated quinoline to a mutagen, but the enzymes from MCA-pretreated DBA mice were not effective for inducing the enzymes that convert quinoline to a mutagen.

Human liver converted quinoline to a mutagen; the activity found in human liver was comparable to that found in a MCA-pretreated rat liver. Tissue preparations from small intestine and lung of other species were inactive, regardless of the pretreatment procedure (data not shown).

In Vitro Inhibition of Quinoline Mutagenicity

The effects of chemicals that inhibited the mutagenicity of quinoline are shown in table 3. The concentrations recorded were the lowest amounts required for maximum reduction of quinoline mutagenicity. The results showed that menadione, an effective uncoupler of the mixed-function oxidases, and α-NF, a potent inhibitor of the P-448-dependent hydroxylase system (*14*), were the most effective inhibitors of quinoline mutagenicity. BHT and vitamin A acetate, which can depress the formation of malignant lesions in rodents by carcinogenic polycyclic hydrocarbons (*15, 17*), were also effective but were less active than menadione or α-NF. Glutathione added in vitro to the Ames assay inhibited quinoline mutagenicity (table 3). Conversely, the liver enzymes from glutathione-depleted rats enhanced quinoline mutagenicity (table 2).

Metabolites of [^{3}H]quinoline Associated With Mutagenesis

Rat liver microsomes converted quinoline to nonpolar

TABLE 2.—*Pretreatment procedure and sources of tissues as factors affecting quinoline mutagenicity*

Stock and strain	Treatment	Tissue	Protein/plate mg	Quinoline/plate μg	TA 100 revertant[a]	Fold increase[b]
Rat, Sprague-Dawley	ARO	Liver	5.0	500	2,552	22
"	"	Lung	1–3	"	74	1
"	"	Intestine	"	"	90	1
"	ARO-DEM	Liver	5.0	"	3,532	31
"	β-NF	"	1.25–2.5	"	692	6
"	MCA	"	2.5–5.0	"	881	8
"	PCN	"	1.25–5.0	"	197	2
"	Quinoline	"	"	"	140	1
"	PB	"	"	"	158	1
"	Control	"	"	"	109	1
Mouse						
C57BL	MCA	"	1.6	200	1,336	12
"	Control	"	"	"	70	1
DBA	MCA	"	"	"	90	1
"	Control	"	"	"	108	1
Human	None	"	4.5	250	525[c]	6
"	None	"	6.0	500	746[c]	8
Rat, Sprague-Dawley	ARO	"	5.0	—	114[d]	1
Human	None	"	7.0	—	91[d]	1

[a] Number of TA 100 revertants at the protein concentration yielding the greatest activation. If values did not differ more than 25% at different protein concentrations, results were averaged.

[b] Number of TA 100 revertants expressed as a multiple of the spontaneous mutation frequency. Each value represents the average of at least two observations.

[c] Single determination.

[d] Average of five determinations.

TABLE 3.—*Inhibitors of quinoline mutagenicity*

Inhibitor	Dose μmoles/plate[a]	Quinoline/plate μg	TA 100 revertants[b]	Percent inhibition[c]
None	—	—	114	—
None	—	500	1,093[d] (1,297)	—
Menadione	5	"	−25 (89)	100+
BHT	25	"	60 (174)	95
Glutathione	25	"	182 (296)	83
Cysteine	12.5	"	1,096 (1,120)	08
Vitamin A	12.5	"	83 (197)	92
Methylmercaptoimidazole	25	"	992 (1,106)	10
α-NF	5	"	63 (117)	94

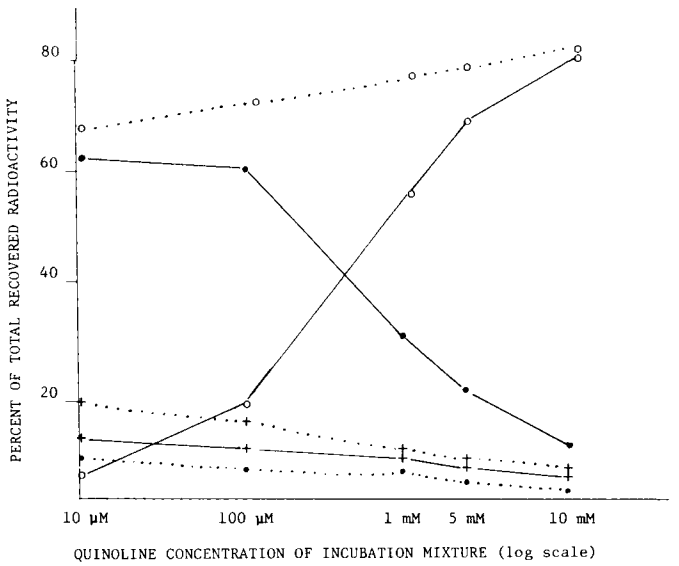
[a] Concentration of inhibitor recorded is the lowest dose at which maximum effect on mutagenicity occurred and no toxicity to the tester strain was observed.

[b] Numbers are average values of two or more observations. The numbers of actual revertants, without subtraction of spontaneous mutation frequency, are *in parentheses*.

[c] Calculated by the net experimental values expressed as a percent of the positive control value.

[d] Control value.

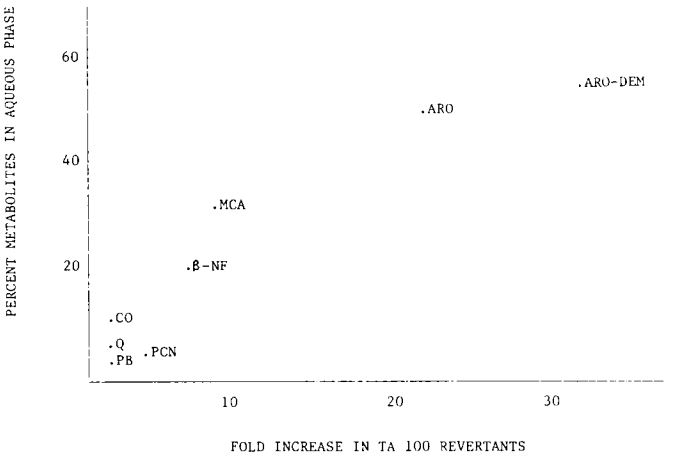
metabolites, which were extracted from an aqueous incubation mixture by chloroform:isoamyl alcohol (17:3) and to water-soluble metabolites that remained in the aqueous phase after extraction. A comparison of metabolites produced by ARO-induced and noninduced microsomes showed that induced microsomes formed much more of the aqueous-soluble metabolites at subsaturating concentrations of quinoline (text-fig. 2). Liver microsomes from MCA- and β-NF-induced S-9 preparations also converted a larger fraction of substrate to aqueous-soluble metabolites, whereas microsomes from PB, PCN, and quinoline-treated rats did not (table 4). As noted previously, these latter three preparations were also ineffective as quinoline activators in the mutagen assay (table 3). The quantitative association between mutagenicity and the formation of quinoline metabolites is shown in text-figure 3. The correlation coefficient (R) for these two parameters was 0.96.



TEXT-FIGURE 2.—Formation of aqueous metabolites of [^{3}H]quinoline by ARO-induced and noninduced S-9. Noninduced S-9 (·····); ARO-induced S-9 (——); unmetabolized quinoline (●); nonpolar metabolites (+); water-soluble metabolites (○).

TABLE 4.—*[^{3}H]Quinoline metabolites formed after incubation with induced liver microsomes*

Fraction	Percent of total recovered radioactivity with inducers:							
	ARO-DEM	ARO	MCA	β-NF	PCN	Quinoline	PB	Control
Quinoline	23	24	50	64	87	88	88	80
Nonpolar metabolite	21	25	15	14	6	5	7	9
Polar metabolite	55	51	34	22	6	6	4	11



TEXT-FIGURE 3.—Aqueous metabolite formation and mutagenic activation of quinoline by various liver S-9 preparations. CO=control; Q=quinoline. R=0.96.

DISCUSSION

The mutagenic and carcinogenic properties of quinoline were recognized only in the past year; detailed information concerning its mechanisms of toxicity are therefore limited. Hirao et al. (*2*) suggested that quinoline-*N*-oxide is the proximal carcinogen formed from quinoline. This possibility seems unlikely because quinoline-*N*-oxide is not mutagenic in the *S. typhimurium* bioassay, a test system in which mutagenicity and carcinogenicity are highly correlated (*10*).

Structure-activity studies show that addition of hydroxy groups at C-5 or C-8 of the quinoline nucleus or the addition of methyl groups at C-4, C-6, C-7, or C-8 [(*18*); reported during the preparation of this manuscript] does not abolish or markedly reduce the TA 100 mutagenic activity. By contrast, methyl or hydroxyl substitution at C-2 and C-3 abolishes the mutagenicity

of quinoline in TA 100, the only strain in which it is expressed. These results suggest that the C-2 and C-3 regions of quinoline are critical sites for the enzymatic conversion of quinoline to its principal mutagenic intermediate. However, some of the quinoline derivatives are weakly mutagenic in other tester strains, which suggests that alternate routes of activation, possibly independent of the C-2 and C-3 regions, may exist for these compounds.

Clues to the chemical nature of the reactive quinoline intermediate may be provided from studies that have examined the metabolism and mutagenicity of benzo[*a*]pyrene, which, like quinoline, is a carcinogenic, unsubstituted polycyclic aromatic hydrocarbon. Benzo[*a*]pyrene is converted to mutagenic epoxides by a P-448-dependent AHH system (*19, 20*). This process is inhibited by BHT, vitamin A, α-NF, and glutathione (*21, 22*) and potentiated by liver preparations from glutathione-depleted rats (*8*). These characteristics also apply to the enzymatic processes that convert quinoline to a mutagen. From the results obtained here, we infer that quinoline is converted to quinoline epoxide(s). The intermediate(s) may then react with nucleic acids to cause mutations in the *S. typhimurium* test strains. Quinoline is mutagenic only in TA 100, and the structure-activity results obtained with this strain strongly suggest that unsubstituted C-2 and C-3 regions are critical for the expression of this mutagenicity. Therefore, the principal mutagen arising from quinoline activation may be quinoline-2,3-epoxide. The correlation of mutagenicity with the formation of water-soluble metabolites and the observation that quinoline is metabolized to dihydrodiols (*23*) are consistent with this conclusion.

The observation that human liver enzymes can convert quinoline to a mutagen suggests that quinoline and its mutagenic derivatives are potential hazards in the industrial environment and that their toxic properties should be reevaluated.

REFERENCES

(*1*) Talcott R, Hollstein M, Wei E: Mutagenicity of 8-hydroxyquinoline and related compounds in the *Salmonella typhimurium* bioassay. Biochem Pharmacol 25:1323–1328, 1976

(*2*) Hirao K, Shinohara Y, Tsuda H, et al: Carcinogenic activity of quinoline on rat liver. Cancer Res 36:329–335, 1976

(*3*) Icke R: Synthesis of *m*-methoxybenzaldehyde. *In* Organic Syntheses, vol 3. New York: Wiley & Sons, 1955, p 564

(*4*) Ames BN, Durston WE, Yamasaki E, et al: Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA 70:2281–2285, 1973

(*5*) Ames BN, McCann J, Yamasaki E: Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 31:347–364, 1975

(*6*) Gornall AG, Bardawill CJ, David MM: Determination of serum proteins by means of the biuret reaction. J Biol Chem 177:751–766, 1949

(*7*) Felton JS, Nebert DW: Mutagenesis of certain activated carcinogens in vitro associated with genetically mediated increases in monooxygenase activity and cytochrome P_1-450. J Biol Chem 250:6769–6778, 1975

(*8*) Haugen D, Coon M, Nebert DW: Induction of multiple forms of mouse liver cytochrome P-450. J Biol Chem 251:1817–1827, 1976

(*9*) Mitchell JR, Jollow DJ, Potter WZ, et al: Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther 187:211–217, 1973

(*10*) McCann J, Choi E, Yamasaki E, et al: Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Natl Acad Sci USA 72:5135–5139, 1975

(*11*) Bowden JP, Chung K-T, Andrews AW: Mutagenic activity of tryptophan metabolites produced by rat intestinal microflora. J Natl Cancer Inst 57:921–924, 1976

(*12*) Lu AY, Kuntzman R., Conney AH: The liver microsomal hydroxylation enzyme system: Induction and properties of the functional components. Front Gastrointest Res 2:1–31, 1976

(*13*) Alvares AP, Bickers DR, Kappas A: Polychlorinated biphenyls: A new type of inducer of cytochrome P-448 in the liver. Proc Natl Acad Sci USA 70:1321–1325, 1973

(*14*) Wiebel FJ, Leutz JC, Diamond L, et al: Aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase in microsomes from rat tissues: Differential inhibition and stimulation by benzoflavones and organic solvents. Arch Biochem Biophys 144:78–86, 1971

(*15*) Wattenberg LW: Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. J Natl Cancer Inst 48:1425–1430, 1972

(*16*) Chu EW, Malmgren RA: An inhibitory effect of vitamin A on the induction of tumors of forestomach and the cervix in the Syrian hamster in carcinogenic polycyclic hydrocarbons. Cancer Res 25: 884–895, 1965

(*17*) Saffiotti U, Montesano R, Sellakumar AR, et al: Experimental cancer of the lung. Inhibition by vitamin A of the induction of tracheobronchial squamous metaplasia and squamous cell tumors. Cancer 20:857–864, 1967

(*18*) Nago M, Yahagi T, Seino U, et al: Mutagenicities of quinoline and its derivatives. Mutat Res 42:335–342, 1977

(*19*) Wood AW, Levin W, Lu AY, et al: Metabolism of benzo[*a*]pyrene and benzo[*a*]pyrene derivatives to mutagenic products by highly purified hepatic microsomal enzymes. J Biol Chem 251:4882–4890, 1976

(*20*) Wood AW, Wislocki PG, Chang RL, et al: Mutagenicity and cytotoxicity of benzo[*a*]pyrene benzo-ring epoxides. Cancer Res 36:3358–3366, 1976

(*21*) Hill DL, Shih TW: Inhibition of benzo[*a*]pyrene metabolism catalyzed by mouse and hamster lung microsomes. Cancer Res 35:2717–2723, 1975

(*22*) ———: Vitamin A compounds and analogs as inhibitors of mixed-function oxidases that metabolize carcinogenic polycyclic hydrocarbons and other compounds. Cancer Res 34:564–570, 1974

(*23*) Posner HS, Mitoma C, Udenfriend S: Enzymic hydroxylation of aromatic compounds. Arch Biochem Biophys 94:280–290, 1961