Biochemical Pharmacology, Vol. 28, pp. 601–606.

BIOCHEM MS. 880/3952

METABOLIC ACTIVATION OF NITROFURANTOIN—POSSIBLE IMPLICATIONS FOR CARCINOGENESIS*

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(*Received* 20 *February* 1978; *accepted* 24 *July* 1978)

Abstract—Although past investigations have indicated that nitrofurantoin is noncarcinogenic, the present studies demonstrate several features of the metabolism of the drug which are similar to those of other nitrofurans that are known carcinogens. Microsomal and soluble fractions from both rat liver and lung mediated the covalent binding of [^{14}C]nitrofurantoin to tissue macromolecules *in vitro*. Oxygen strongly inhibited the binding in both the microsomal and soluble fractions, and carbon monoxide failed to inhibit binding in microsomal preparations, indicating nitrofurantoin was activated in both systems by nitroreduction and not by oxidation of the furan ring. An antibody against NADPH-cytochrome *c* reductase inhibited the microsomal nitroreduction and covalent binding of nitrofurantoin, while the addition of a flavin (FAD) markedly enhanced the covalent binding. Maximal rates of covalent binding were obtained in soluble fractions in the presence of NADH or hypoxanthine; covalent binding was inhibited in these fractions by allopurinol, an inhibitor of xanthine oxidase. Nitroreduction of nitrofurantoin was enhanced, but covalent binding was decreased, in liver microsomes from phenobarbital-pretreated rats. Phenobarbital did not alter nitroreduction or covalent binding of nitrofurantoin in lung microsomes or in soluble fractions from lung or liver. Reduced glutathione markedly decreased covalent binding of nitrofurantoin, in both the microsomal and the soluble fractions from liver and lung, but did not alter the rate of nitroreduction in any of the fractions. Radioactivity was covalently bound in several organs of rats given [^{14}C]nitrofurantoin *in vivo*.

It is now recognized generally that many types of adverse reactions to drugs and other chemicals are due to the metabolism of the parent compounds to highly reactive chemical species. These toxic metabolites may disrupt the structure and function of tissue macromolecules in various ways, such as by alkylation of proteins and nucleic acids or by stimulation of lipid peroxidation, and thereby result in a variety of pathological responses including cellular necrosis and carcinogenesis [1–4].

Furan derivatives containing a 5-nitro substituent are known to be reduced *in vitro* by cytosol enzymes such as aldehyde oxidase [5] and xanthine oxidase [6–10], or by flavin-containing microsomal enzymes such as cytochrome *c* reductase [10]. Enzymatic reduction of some nitrofurans has been shown to produce highly reactive metabolites capable of covalently binding to tissue macromolecules [10–12]. The reactive metabolites are suspected to be *N*-hydroxylaminofurans, which are thought to be responsible for the carcinogenicity of these and numerous other nitrofurans *in vivo* [10–12]. Interestingly, the widely used antibacterial agent, nitrofurantoin (*N*-[5-nitro-2-furfurylidine]-1-aminohydantoin) (NF), is reportedly noncarcinogenic [13, 14] and therefore, possibly unique among the nitrofuran series. Some investigators have speculated that nitrofurantoin may be noncarcinogenic because it is not metabolized to an hydroxylamine or to other potentially carcinogenic metabolites [15]. Past studies have not explored this hypothesis directly, although Wang *et al.* [10] showed that nitrofurantoin could be reduced under anaerobic conditions *in vitro* (as measured by the loss of nitro group absorbance at 400 nm) by xanthine oxidase and NADPH-cytochrome *c* reductase, and Rosenkranz and Speck [16] have suggested that nitrofurantoin could be reduced enzymatically to a mutagenic metabolite under anaerobic conditions.

Mitchell *et al.* [17] reported the production of liver and kidney necrosis in experimental animals by administration of several different furan derivatives. Boyd [18] described another furan, 4-ipomeanol, which produced selective lung toxicity in several species. Similarly, the work of Swenson *et al.* [19] suggests that the dihydrofuran moiety of aflatoxin B_1 is responsible for the hepatocarcinogenicity of this substance. All of these toxicities appear to be due to oxygen-dependent metabolic activation of the compounds by cytochrome P-450-dependent mixed-function oxidases [17–19]. The proximate toxic or carcinogenic metabolites are suspected to be the corresponding furan-epoxides, formed from oxidation of the furan ring.

The present investigation was undertaken to determine whether nitrofurantoin, like other furans and nitrofurans, is enzymatically converted either aerobically or anaerobically to metabolites sufficiently reactive to alkylate tissue macromolecules.

MATERIAL AND METHODS

Experimental animals. Male, HLA-SD rats weighing 150 g were obtained from Hilltop, Inc., Scottdale, PA. Animals were fed standard rat chow and water *ad lib.*, and were not fasted prior to use.

Phenobarbital pretreatment. In some experiments

* Presented in part at the Annual Meeting of the Federation of American Societies of Experimental Biology, Atlantic City, NJ, April 1975.

animals were pretreated with phenobarbital, prior to the removal of the liver and lungs. The pretreatment consisted of twice daily injections of sodium phenobarbital (50 mg/kg) for 5 days. The last dose was given 24 hr prior to removal of the organs.

Radiolabeled substrates. Nitrofurantoin[methylene-^{14}C] was the gift of Norwich Pharmacal Co., (Norwich, NY) or was purchased from Pathfinder Laboratories, Inc. (St. Louis, MO). 4-Ipomeanol-[3, 5-^{14}C] was synthesized as described previously [20]. For *in vitro* experiments, specific activities were adjusted to 200–300 dis./min/nmole by addition of the unlabeled compounds. [^{14}C]nitrofurantoin administered *in vivo* had a specific activity of 2200 dis./min/nmole. Chemical and radiochemical purities of both radiolabeled compounds were >99 per cent.

Other chemicals. The reduced nucleotides, NADPH (nicotinamide adenine dinucleotide phosphate) and NADH (nicotinamide adenine dinucleotide), and *N*-methylnicotinamide, hypoxanthine, and reduced glutathione were all obtained from Sigma Chemical Co. (St. Louis, MO).

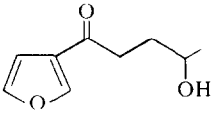
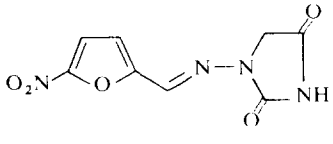
In vitro *incubations.* Microsomal and soluble-fraction preparations were obtained from lung and liver by methods described previously [21]. Incubations were run in septum-stoppered 30-ml Erlenmeyer flasks at 37°. Gases were bubbled through the microsomal suspensions for 5 min prior to use. Microsomal and soluble-fraction proteins were adjusted to final concentrations of 2 mg/ml and 4 mg/ml, respectively, in a total volume of 2 ml of 0.05 M Hepes buffer (pH 7.8) per incubation. Reactions were started by addition of the appropriate substrate (in 5 μl of dimethylsulfoxide) and cofactor(s) and were terminated by addition of 2 ml of 20% trichloroacetic acid (TCA). Other experimental details are given in the figure legends.

Measurements of covalently bound metabolites and total nitroreduction. Covalently bound radioactivity was estimated in trichloroacetic acid (20%)-precipitated enzyme suspensions or whole-tissue homogenates (1:4, w:v, aqueous) as an index of the formation of chemically reactive metabolites. The precipitates were washed twice with water, twice with 50% methanol/water, and finally ten times with 100% methanol at 50° to remove unbound radioactivity. That the radioactivity remaining bound to the precipitates after the wash procedure was covalently bound was confirmed by the failure of several additional procedures, including Sephadex chromatography, dialysis, and repeated solubilization in 1 N NaOH followed by reprecipitation, to remove additional radioactivity. The radiolabeled precipitates were solubilized in 1 N NaOH and radioactivity was determined in aliquots by liquid scintillation counting. Protein was quantitated by a biuret method [22], using bovine serum albumin as a stand ard, and covalently bound radioactivity was expressed as pmoles or nmoles bound/mg of protein. Total nitroreduction was measured as described by Wang *et al.* [10] by the loss of absorbance at 400 nm, in the supernatant solutions from the trichloroacetic acid precipitation. NADPH-cytochrome *c* reductase was measured by the method of Williams and Kamin [23].

RESULTS

Since the furan derivative, 4-ipomeanol, is known to be activated metabolically by both hepatic and pulmonary mixed-function oxidases [18], we compared the covalent binding of this compound with that of nitrofurantoin. The data shown in Table 1 indicate that both 4-ipomeanol and nitrofurantoin were converted to reactive metabolites in rat liver and lung microsomes, but that the respective metabolic reactions were quite different. Whereas, in the presence of NADPH, maximal covalent binding of 4-ipomeanol occurred in the presence of air, the greatest binding of nitrofurantoin occurred under anaerobic conditions. Carbon monoxide strongly inhibited activation of 4-ipomeanol, but the binding of nitrofurantoin was actually greatest under a pure carbon monoxide atmosphere. The findings with

Table 1. Comparison of covalent binding of [^{14}C]-4-ipomeanol and [^{14}C]nitrofurantoin in rat liver and lung microsomes with different incubation atmospheres

Compound	Incubation atmosphere	Covalent binding *† Liver	Lung
 4-Ipomeanol	Air	5.6	4.8
	O_2:CO (20:80)	0.8	0.5
	N_2:CO (20:80)	0.1	0.1
	CO	0.1	0.1
	N_2	0.1	0.1
 Nitrofurantoin	Air	0.7	0.2
	O_2:CO (20:80)	0.9	0.2
	N_2:CO (20:80)	10.5	9.0
	CO	11.1	9.1
	N_2	10.8	9.1

* Values shown are expressed as nmoles bound/mg microsomal protein/5-min incubation and are means of five determinations. Standard errors (not shown) were all less than 6 per cent of the respective means.

† All incubations contained NADPH (2×10^{-3} M).

Table 2. Effect of NADPH and FAD on covalent binding of [^{14}C]nitrofurantoin in rat liver and lung microsomes*

NADPH present†	FAD present†	Covalent binding‡ Liver	Lung
+	–	10.0	6.6
–	–	0.2	0.1
+	+	19.7	14.1
–	+	0.2	0.2

* All incubations were run anaerobically, under N_2.
† NADPH and FAD concentrations were 2×10^{-3} M.
‡ Values are expressed as nmoles bound/mg microsomal protein/5-min incubation, and are the means of five determinations. Standard errors were all less than 6 per cent of the respective means.

4-ipomeanol are consistent with earlier studies which demonstrated the metabolic activation of the furan moiety of the compound by pulmonary and hepatic microsomal cytochrome P-450-dependent mixed-function oxidases [18]. In contrast, increased binding by nitrofurantoin under anaerobic conditions and failure of carbon monoxide to inhibit binding suggest that microsomal activation of nitrofurantoin occurs by a reductive process and not by oxidation of the furan ring. The results with carbon monoxide also indicate that cytochrome P-450 is not required for the activation of nitrofurantoin by liver or lung microsomes.

Table 2 shows that very little covalent binding of nitrofurantoin occurred in microsomes when NADPH was deleted. A similarly low level of binding was observed when boiled microsomes were incubated in the presence or absence of NADPH (data not shown).

In the presence of NADPH, the addition of a flavin (FAD) markedly stimulated covalent binding of nitrofurantoin (Table 2). No stimulation was obtained with FAD when NADPH was deleted, suggesting that reduced flavin was involved in the stimulation of nitrofurantoin binding in microsomes, similar to the findings reported by Kamm and Gillette [24] for the reduction of other types of nitro compounds. The microsomal enzyme which activated nitrofurantoin thus had characteristics which resembled NADPH-cytochrome *c* reductase. This was further indicated by the inhibitory effects on reduction and covalent binding of nitrofurantoin by addition to the microsomal incubations of an antibody (prepared as described previously [25]) against purified NADPH-cytochrome *c* reductase (Table 3).

Table 3. Effect of an antibody against NADPH-cytochrome *c* reductase on nitroreduction and covalent binding of [^{14}C]nitrofurantoin in rat liver and lung microsomes*

Source of microsomes	Antibody present†	Nitroreduction‡	Covalent binding§
Liver	–	50	4.8
	+	31‖	1.8‖
Lung	–	27	1.7
	+	12‖	0.8‖

* All incubations were run anaerobically, under an N_2 atmosphere, in the presence of 2×10^{-3} M NADPH. Values shown are means of triplicate determinations; standard errors were all less than 3 per cent of the respective means.
† Protein concentrations in incubations not containing antibody were matched to those with antibody by addition of preimmune globulin having no anti-cytochrome *c* reductase activity. In incubations containing the antibody, NADPH-cytochrome *c* reductase activities were 21 and 22 per cent of the control values in liver and lung microsomes respectively.
‡ Expressed as nmoles reduced/mg of protein/5-min incubation.
§ Expressed as nmoles bound/mg of protein/5-min incubation.
‖ Significantly different ($P < 0.01$) than control value, using Student's *t*-test.

Table 4. Cofactor requirements for covalent binding of [^{14}C]nitrofurantoin in soluble-fraction preparations from rat liver and lung*

Cofactor(s) present†	Covalent binding‡ Liver	Lung
NADH	12.9	3.8
NADPH	5.0	0.3
N-methylnicotinamide	0.6	0.1
Hypoxanthine	11.5	2.5
Mixture of all above	14.0	5.0

* Endogenous cofactors and reduced glutathione were removed from the preparations by rapid passage through a 10 × 60 cm column of Sephadex G-25. The enzyme preparation eluted with the column void column while the other components were completely retained on the column. This was confirmed by preliminary experiments which showed negligible nitroreduction or covalent binding of nitrofurantoin, without the addition of cofactors. Similarly, negligible binding was also obtained using boiled preparations, with or without the presence of the cofactors. All incubations were run anaerobically, under N_2.
† All cofactor concentrations were 1×10^{-3} M except hypoxanthine which was 0.25×10^{-3} M (due to its low solubility).
‡ Values are expressed as nmoles bound/mg of protein/5-min incubation and are the means of triplicate determinations. Standard errors were all less than 2 per cent of the respective means.

The data in Table 4 indicate that soluble-fraction enzymes of both lung and liver could also convert nitrofurantoin to a reactive metabolite under anaerobic conditions. Maximal levels of covalent binding were obtained in soluble-fraction preparations only in the presence of NADH or hypoxanthine. Little additional binding was seen with the addition of *N*-methylnicotinamide or NADPH, suggesting that activation in these preparations was due principally to a soluble enzyme with the characteristics of xanthine oxidase, with little if any contribution by aldehyde oxidase. Binding was strongly inhibited (data not shown) by oxygen in all of the soluble-fraction preparations, as well as by 10^{-3} M allopurinol (>50 per cent inhibition), an inhibitor of xanthine oxidase.

Table 5 shows that pretreatment of the rats with phenobarbital, an inducer of numerous pathways of drug metabolism, affected only the liver microsomal metabolism of nitrofurantoin. As expected, the rate of nitroreduction increased parallel to the increased activity of NADPH-cytochrome *c* reductase in phenobarbital-induced rat liver microsomes. However, the amount of covalently bound nitrofurantoin was significantly

Table 5. Nitroreduction and covalent binding of [^{14}C]nitrofurantoin in microsomal and soluble-fraction preparations from livers and lungs of rats pretreated with saline or phenobarbital*

		Nitroreduction†		Covalent binding‡		NADPH-cytochrome *c* reductase activity§	
Pretreatment	Fraction	Liver	Lung	Liver	Lung	Liver	Lung
Saline	Microsomal	57	27	10.2	8.0	112	47
	Soluble	26	7	13.3	5.2		
Phenobarbital	Microsomal	72‖	25	8.0‖	7.9	164‖	48
	Soluble	26	7	13.4	5.3		

* All incubations were run anaerobically, under an N_2 atmosphere. Microsomal incubations were run in the presence of 2×10^{-3} M NADPH; soluble-fraction preparations contained a mixture of cofactors consisting of NADH (10^{-3} M), *N*-methylnicotinamide (10^{-3}M), and hypoxanthine (0.25×10^{-3}M). Values shown are means of triplicate determinations. Standard errors were all less then 7 per cent of the respective means.

† Expressed as nmoles reduced/mg of protein/5-min incubation.

‡ Expressed as nmoles bound/mg of protein/5-min incubation.

§ Expressed as nmoles cytochrome *c* reduced/mg of protein/min.

‖ Significantly different ($P < 0.01$) from control value, using Student's *t*-test.

Table 6. Effect of GSH on nitroreduction and covalent binding of [^{14}C]nitrofurantoin in microsomal and soluble fraction preparations from rat liver and lung*

		Nitroreduction†		Covalent binding‡		NADPH-cytochrome *c* reductase activity§	
Fraction	GSH (10^{-3} M) present	Liver	Lung	Liver	Lung	Liver	Lung
Microsomal	–	62	27	10.5	8.1	113	47
	+	63	27	4.7‖	1.1‖	120	50
Soluble	–	25	8	13.8	5.5		
	+	24	9	2.6‖	1.0‖		

* All incubations were run anaerobically, under an N_2 atmosphere. Microsomal incubations were run in the presence of 2×10^{-3}M NADPH; soluble-fraction preparations contained a mixture of cofactors consisting of NADH (10^{-3} M), *N*-methylnicotinamide (10^{-3} M), and hypoxanthine (0.25×10^{-3} M). Values shown are means of triplicate determinations; standard errors were all less than 3 per cent of the respective means.

† Expressed as nmoles reduced/mg of protein/5-min incubation.

‡ Expressed as nmoles bound/mg of protein/5-min incubation.

§ Expressed as nmoles cytochrome *c* reduced/mg of protein/min.

‖ Significantly ($P < 0.01$) different from corresponding control values, using Student's *t* test.

Table 7. Covalent binding of [^{14}C]nitrofurantoin in tissues of the rat after intravenous administration of various doses*

	Normal rats		Antibiotic-pretreated rats†	
Tissue	1.5 mg/kg	15 mg/kg	90 mg/kg	(90 mg/kg)
Kidney	6.2 ± 1.2	53.7 ± 10.8	609 ± 12	785 ± 2
Liver	2.0 ± 0.8	20.8 ± 5.7	258 ± 14	269 ± 6
Ileum	2.5 ± 1.0	28.3 ± 2.3	237 ± 93	256 ± 14
Lung	ND‡	9.0 ± 1.0	174 ± 4	198 ± 36
Heart	ND	6.5 ± 1.0	109 ± 31	125 ± 30
Pancreas	ND	ND	100 ± 14	ND
Blood	ND	ND	97 ± 10	ND
Spleen	ND	11.0 ± 2.1	75 ± 5	ND
Muscle	ND	9.5 ± 3.6	52 ± 1	58 ± 4
Testes	ND	9.6 ± 4.0	18 ± 2	ND
Brain	ND	1.5 ± 0.5	10 ± 2	ND

* Nitrofurantoin (sodium salt; sp. act. 1.0 mCi/m-mole) was given via the femoral vein as a solution in 0.25 ml of rat plasma containing 0.5% sodium citrate as an anticoagulant. The rats were lightly anesthetized with ether during administration of the drug. Rats were killed 24 hr after administration of the drug. Results are expressed as pmoles bound/mg of tissue protein and are the means of determinations on groups of six animals each.

† Each rat was given (by intragastric intubation) an aqueous suspension of a mixture of 50 mg neomycin, 25 mg tetracycline, and 25 mg bacitracin, in a total volume of 0.5 ml, twice daily for 3 days.

‡ Not determined.

less in the induced microsomes. This suggests that the rate-limiting steps for microsomal alkylation and for the complete nitroreduction of nitrofurantoin are different in hepatic microsomes from phenobarbital-induced rats. Phenobarbital pretreatment had no effect on either the pulmonary microsomal cytochrome *c* reductase activity or the total reduction or covalent binding of nitrofurantoin in lung microsomes. Also, the pretreatment did not affect the total reduction or the covalent binding in soluble-fraction preparations from either liver or lung.

With both the microsomal and soluble-enzyme preparations, the addition of the nucleophilic tripeptide, reduced glutathione (GSH), did not inhibit the reduction of nitrofurantoin, but markedly inhibited the covalent binding (Table 6). The failure of added GSH to affect the amount of unreduced nitrofurantoin remaining in the incubation mixtures (measured by nitro group absorption at 400 nm) also suggested that little, if any, of the parent drug was lost due to direct conjugation with GSH, a reaction known to occur with some nitrofurans [26]. These studies suggested that an alkylating metabolite formed from reduction of nitrofurantoin was highly electrophilic and could react with nucleophilic sites on tissue macromolecules, or alternatively, with mobile nucleophiles such as GSH, to form less reactive conjugates and thereby prevent the covalent binding to macromolecules.

When a range of doses of radiolabeled nitrofurantoin was given intravenously to rats, radioactivity became covalently bound to several tissues, especially the kidney, liver, ileum and lung (Table 7), suggesting that the drug is also metabolically activated *in vivo*. Certain common gut bacteria are known to have the capacity to reduce some nitrofurans [27]. But, pretreatment of rats with an antibiotic regimen to decrease the microbial flora of the gut [28] did not decrease the amount of radioactivity bound to tissues *in vivo*, indicating that the bound radioactivity was probably not due to reactive metabolites derived from microbial metabolism of nitrofurantoin.

DISCUSSION

These studies indicate that nitrofurantoin is reduced to highly reactive metabolite(s) by enzymes present in both the microsomal and soluble fractions obtained from homogenates of rat liver and lung. Therefore, it appears unlikely that the presumed lack of carcinogenicity of nitrofurantoin is due to its inability to be activated to metabolite(s) capable of alkylating biological macromolecules.

That the drug is activated primarily by a reductive transformation and not by oxidation of the furan ring was demonstrated by the comparative metabolic studies with the furan derivative, 4-ipomeanol. This conclusion is supported further by the observations that the acute hepatic, renal, and pulmonary cellular necroses produced by electophilic metabolites of many furan derivatives [17, 18, 29] are not seen with nitrofurantoin [30]. Nitrofurantoin may produce pulmonary damage in humans [31] and in rats [30], but pulmonary Clara cells do not appear to be the primary cellular target. Clara cells are especially susceptible to the toxicity of compounds, such as 4-ipomeanol, that require oxidative activation [29]. Although radioactivity is covalently bound *in vivo* in rats after administration of radiolabeled nitrofurantoin (Table 7), it is not preferentially bound to the lungs, as it is with 4-ipomeanol [18, 29].

The hepatic and pulmonary microsomal enzymes that activate nitrofurantoin appear to closely resemble NADPH-cytochrome *c* reductase, by their requirements for NADPH, stimulation by a flavin, and inhibition by an antibody prepared against the purified enzyme. Wang *et al.* [10] previously have shown that several nitrofurans, including nitrofurantoin, were reduced (measured by loss of 400 nm absorbance) by a partially purified NADPH-cytochrome *c* reductase from rat liver. Buege and Aust [32] have shown that liver and lung contain NADPH-cytochrome *c* reductases that are very similar, as assessed by immunochemical methods.

Based on the requirement for NADH or hypoxanthine, the soluble-fraction enzymes of lung and liver that activate nitrofurantoin most closely resemble xanthine oxidase. This is further supported by the finding of Wang *et al.* [10] that nitrofurantoin was reduced by purified milk xanthine oxidase. Xanthine oxidase is known to be present in several rat tissues including liver and lung [33].

The actual chemical species, formed during the reduction of nitrofurantoin, which alkylates tissue macromolecules has not been identified due to its extreme lability, but available precedent suggests it may be a hydroxylaminofuran or, possibly, a nitroso-derivative. Others have speculated that nitrofurans may be reduced to hydroxylaminofurans, based on stoichiometric considerations or by analogy with the evidence for hydroxylamine formation from other types of nitro compounds [9–16, 34, 35]. It also has been suggested that, analogous to the reduced products of aromatic nitro compounds [36–38], enzymatically reduced metabolites of nitrofurans may be capable of binding to macromolecules [9–12]. Hydroxylamines have been strongly advocated as proximate carcinogenic metabolites of arylamines [1], and it is reasonable to speculate that they may play a similar role for carcinogenic nitrofurans.

The oxygen sensitivity of the enzymatic activation of nitrofurantoin which we observed in the present experiments may be related to the extreme oxygen lability of an intermediate hydroxylamino derivative [39]. An alternative explanation, however, derives from recent studies by Mason and Holtzman [40] who have proposed another mechanism for the oxygen sensitivity of nitroreductase. The first step in the enzymatic reduction of certain nitro compounds, including nitrofurantoin [41], was shown to be the transfer of a single electron to the substrate to generate a nitroaromatic anion radical. Certain nitroaromatic anion radicals presumably can react with oxygen to produce superoxide and regenerate the parent nitro compound [40, 41]. Thus, under aerobic conditions the cyclic reduction/oxidation of the parent compound would generate superoxide continuously, but prevent the formation of partially reduced products which could bind covalently to macromolecules, and would also inhibit the formation of fully reduced products such as the corresponding amines or, specifically in the case of nitrofurantoin, possibly open-chain nitrile derivatives as recently described by Aufrère *et al.* [42].

While the *in vivo* binding of radioactivity after administration of [^{14}C]nitrofurantoin does not appear to be associated with the production of acute cellular necrosis, as it is with some furan derivatives, it does suggest that highly reactive and possibly carcinogenic metabolites of nitrofurantoin may be produced *in vivo*. Published studies evaluating the possible carcinogenicity of nitrofurantoin are quite limited, as has been pointed out by other investigators [16, 43, 44]. Moreover, nitrofurantoin, like known carcinogenic nitrofurans, is mutagenic and has DNA-modifying activity in bacterial test systems [44, 45]. In bacterial testor strains lacking nitroreductase activity, mutagenicity was obtained with nitrofurantoin only after addition of a liver microsomal preparation, and it was enhanced by anaerobiasis [16]. These findings suggested the presence in rat liver of an enzyme activity capable of transforming nitrofurantoin into an active mutagen. Another report indicated that the urine of rats fed nitrofurantoin had mutagenic activity [43]. Based on these reports, and our present studies, we strongly support the suggestions [16, 43, 44] that the potential carcinogenicity of nitrofurantoin should be carefully re-examined, particularly in view of its widespread use in patients with relatively minor ailments. If, on the other hand, nitrofurantoin does prove to be noncarcinogenic, then interesting and important questions will be apparent as to the interrelations of the metabolism, mutagenicity and carcinogenicity of the nitrofurans.

Acknowledgements—We thank Drs. J. R. Gillette and B. A. Chabner for their many helpful comments during these studies and on the manuscript.

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