Metabolism of 2,4-Dinitrotoluene and 2,6-Dinitrotoluene, and Their Dinitrobenzyl Alcohols and Dinitrobenzaldehydes by Wistar and Sprague-Dawley Rat Liver Microsomal and Cytosol Fractions

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The metabolism of 2,4-dinitrotoluene (2,4-DNT), 2,4-dinitrobenzyl alcohol (2,4-DNB), 2,4-dinitrobenzaldehyde (2,4-DNBAl), 2,6-DNT, 2,6-DNB and 2,6-DNBAl in the microsomal and cytosol fractions prepared from unfortified male Wistar and male Sprague-Dawley (S.D.) rat livers was investigated. Data obtained by high-performance liquid chromatography (HPLC) indicated that the products of dinitrotoluenes (2,4-DNT and 2,6-DNT), dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB), and dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) in the microsomal and cytosol preparations containing nicotinamide adenine dinucleotide phosphate (NAD(P)) and reduced NAD(P) (NAD(P)H) were dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB), dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl), and dinitrobenzoic acids (2,4-DNBA and 2,6-DNBA), and dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB), respectively. From these results, it was concluded that the dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) were intermediates in the oxidations of dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB) to dinitrobenzoic acids (2,4-DNBA and 2,6-DNBA), and that the oxidations of dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB) to dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) and the reductions of dinitrobenzaldehydes to dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB) were reversible. The result of the consecutive oxidations of 2,6-DNT in male Wistar rat livers, in the presence of various inhibitors suggests that oxidation of 2,6-DNT to 2,6-DNB is done mainly by microsomal cytochrome P-450, oxidation of 2,6-DNB to 2,6-DNBAl is mediated by microsomal cytochrome P-450 and nicotinamide adenine dinucleotide (NAD)-dependent alcohol dehydrogenase, oxidation of 2,6-DNBAl to 2,6-DNBA may be mediated by NAD-dependent aldehyde oxidase, and reduction of 2,6-DNBAl to 2,6-DNB may be mediated by reduced NAD (NADH)-dependent aldehyde reductase. From the comparative investigation of these reaction activities, it was found that: (a) the activity in the 2,6-DNT oxidation to 2,6-DNB was higher than that in the 2,4-DNT oxidation to 2,4-DNB in both strains, and the activity in Wistar rat was higher than that in S.D. rat; (b) the activities for the reductions of the dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) to dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB) were the highest, among the reactions examined, in both strains, and the reduction activity of 2,4-DNBAl to 2,4-DNB in Wistar rat was particularly high; (c) the activity for 2,6-DNB oxidation to 2,6-DNBAl was higher than that for 2,4-DNB oxidation to 2,4-DNBAl in both strains, and the activity in Wistar rat was higher than that in S.D. rat; (d) the activity for 2,6-DNBAl oxidation to 2,6-DNBA was much less than that for 2,4-DNBAl oxidation to 2,4-DNBA in both strains, and in particular, the activity for oxidation of 2,6-DNBAl to 2,6-DNBA in Wistar rat was low. The present results indicate that the metabolism of DNT isomers differs in different strains of rat.

Keywords dinitrotoluene; dinitrobenzyl alcohol; dinitrobenzaldehyde; dinitrobenzoic acid; isomer; hepatic metabolism; rat; strain difference

We have reported that 2,4-dinitrotoluene (2,4-DNT), an important industrial material, is stepwise metabolized to 2,4-dinitrobenzyl alcohol (2,4-DNB), 2,4-dinitrobenzaldehyde (2,4-DNBAl) and 2,4-dinitrobenzoic acid (2,4-DNBA) in the liver microsomal and cytosol fractions from male Sprague-Dawley (S. D.) rats fortified with phenobarbital and 5,6-benzoflavone, and that the production of 2,4-DNBAl from 2,4-DNB and production of 2,4-DNB from 2,4-DNBAl are reversible.¹⁾ In addition, it has been shown that the oxidation of 2,4-DNT to 2,4-DNB is mediated by reduced nicotinamide adenine dinucleotide (NADPH)-dependent microsomal cytochrome P-450; the oxidation of 2,4-DNB to 2,4-DNBAl is mediated mainly by NADPH-dependent microsomal cytochrome P-450 and nicotinamide adenine dinucleotide (NAD)-dependent cytosolic alcohol dehydrogenases; the oxidation of 2,4-DNBAl to 2,4-DNBA is mainly dependent on the microsomal preparations containing NAD phosphate (NADP) or reduced NAD (NADH) and cytosol preparations containing NADP or NADH, and the reduction of 2,4-DNBAl to 2,4-DNB is mainly dependent on the microsomal preparations containing NAD or NADH.¹⁾ However, the metabolism of 2,4-DNT in unfortified male Wistar rat livers has not been examined. 2,6-DNT, which is an isomer of 2,4-DNT, has been shown to be metabolized stepwise to 2,6-DNB, 2,6-

DNBAl and 2,6-DNBA in unfortified male Wistar rat livers.²⁾ However, the nature of the enzymes participating in the consecutive metabolic reactions of 2,6-DNT was not examined.

In the present study, we have examined the oxidations of methyl groups of 2,4-DNT and 2,6-DNT in the liver microsomal and cytosol fractions from unfortified male Wistar and S.D. rats, in order to explore the strain difference and structural difference in the hepatic metabolism of 2,4-DNT and 2,6-DNT. The metabolism of 2,6-DNT in Wistar rat livers previously reported²⁾ was also re-examined under the same experimental conditions. Furthermore, the nature of the enzymes responsible for the consecutive oxidations of 2,6-DNT in male Wistar rat livers was examined using various inhibitors under air, nitrogen and 80% of CO in oxygen.

Experimental

Materials 2,4-DNT, 2,6-DNT, 2,4-DNBA, phenylhydrazine hydrochloride and *o*-phenanthroline were obtained from Wako Pure Chemical Industries Ltd. 2,4-DNBAl and 2,6-DNBAl were obtained from Aldrich Chemical Co. Hydralazine was obtained from Sigma Chemical Co. Tetra-*n*-butylammonium chloride (TBACl) was obtained from Nakarai Chemicals Ltd. NADPH, NADH, NADP, NAD and alcohol dehydrogenase (345 IU/mg protein, from yeast) were obtained from Oriental Yeast Co., Ltd. β -Diethylaminoethyl diphenyl-*n*-propylacetate hydroch-

loride (SKF-525 A) was a gift from Prof. H. Yoshimura, Kyushu University (Japan). 2,6-DNB and 2,6-DNBA were prepared from 2,6-DNBAl by reduction with NaBH_4 and by oxidation with KMnO_4 , respectively, as previously described.³⁾ 2,4-DNBAl-phenylhydrazine (2,4-DNBAl-Ph)⁴⁾ and 2,6-DNBAl-phenylhydrazine (2,6-DNBAl-Ph)²⁾ were prepared from 2,4-DNBAl and 2,6-DNBAl by reaction with phenylhydrazine as previously described. 2,4-DNB (5.1 g, mp 64°C) was prepared by reacting 2,4-DNBAl (0.05 mol, 10 g) with NaBH_4 (0.08 mol, 2.9 g) in methanol (60 ml) at room temperature for 1 h. *Anal.* Calcd for $\text{C}_7\text{H}_6\text{N}_2\text{O}_3$: C, 42.43; H, 3.05; N, 14.14. Found: C, 42.42; H, 3.03; N, 14.36. *MS* m/z : 198 (M^+), 181, 134. $^1\text{H-NMR}$ [in $(\text{CD}_3)_2\text{SO}$] δ : 4.96 (2H, d, $J=4.0$ Hz, CH_2), 5.88 (1H, br, OH), 8.16 (1H, d, $J=9.0$ Hz, aromatic 6-H), 8.62 (1H, d, $J=2.0$ Hz, aromatic 5-H), 8.80 (1H, d, $J=2.0$ Hz, aromatic 3-H). Solvents used were of the highest grade commercially available.

Preparation of Liver Fractions Male Wistar rats (180–200 g) and male S. D. rats (180–200 g) were obtained from Sankyo Laboratories Co. Animals were killed by decapitation. The livers were removed, minced and homogenized in three volumes of ice-cold 1.15% KCl. The microsomal and cytosol fractions were prepared by differential centrifugation as previously described.¹⁾ Protein was determined by the method of Lowry *et al.*⁵⁾ with bovine serum albumin as a standard.

Incubation of Enzyme Preparations with 2,4-DNT and 2,6-DNT, and Their Benzyl Alcohols and Benzaldehydes Standard incubation mixtures (2 ml) contained 65 mM phosphate buffer (pH, 7.4), 4 mM cofactors (NAD, NADP, NADH or NADPH in H_2O), 1 mM substrates (2,4-DNT, 2,6-DNT, 2,4-DNB, 2,6-DNB, 2,4-DNBAl or 2,6-DNBAl in acetone) and 1.02 or 2.0 mg of microsomal or cytosol proteins. In some experiments, 0.1 to 2 mM inhibitor (SKF-525 A, hydralazine, *o*-phenanthroline) was added. The incubations were carried out at 37°C for 5 to 60 min under air, nitrogen or an atmosphere of 80% of CO in oxygen. The CO-O_2 mixtures were prepared in gas burettes and passed into Thunberg vessels containing the reaction mixtures as previously described.¹⁾ The incubations were terminated by addition of 0.2 ml of 0.5 M HCl followed by the addition of 10 ml of ether. The incubation mixtures were extracted three times with 10 ml of ether. The ether layers were dried over anhydrous Na_2SO_4 , the solvents were evaporated off under an N_2 stream, and the residues were reconstituted in 5 ml of methanol. An appropriate volume (10 μl) of this solution was subjected to high-performance liquid chromatography (HPLC).

Treatment of Ether Extracts with Phenylhydrazine The phenylhydrazine derivatizations of samples containing 2,4-DNBAl⁴⁾ or 2,6-DNBAl²⁾ with phenylhydrazine were carried out according to the methods previously described. A solution of phenylhydrazine hydrochloride (0.5 mg) in methanol (0.15 ml) was added to the residues from the ethereal extracts obtained by incubating 2,4-DNB or 2,6-DNB with microsomal and cytosol preparations. The mixtures were heated for 10 min at 70°C. After diluting the mixture with methanol, an appropriate volume (10 μl) was subjected to HPLC analysis.

HPLC Analysis of Metabolites Produced in the Metabolism of 2,4-DNT, 2,4-DNB or 2,4-DNBAl, and 2,6-DNT, 2,6-DNB or 2,6-DNBAl Ten microliters of the methanolic solutions from ethereal extracts and derivatized samples was injected into a Hitachi model 655A high-performance liquid chromatograph equipped with a reversed-phase column of TSK-gel ODS-80TM (150 $\times$ 4.6 mm i.d., particle size 5 μm , Toyo Soda Kogyo Co.). The mobile phases of 0.05% TBACl in aqueous 40% methanol and aqueous 60% acetonitrile were used for the analysis of metabolites and derivatized samples, respectively. The column temperature was ambient and the flow rate was 1 ml/min. The eluate was monitored by a multi-wave length ultraviolet monitor (Hitachi model 655) at 250 nm. Under these conditions, 2,4-DNT, 2,4-DNB, 2,4-DNBAl and 2,4-DNBA gave retention times of 20.08, 7.66, 10.53 and 6.30; 2,6-DNT, 2,6-DNB, 2,6-DNBAl and 2,6-DNBA gave retention times of 19.98, 3.86, 5.53 and 3.03; 2,4-DNBAl-Ph and 2,6-DNBAl-Ph gave retention times of 11.24 and 7.18. Identification of metabolites was carried out by the co-chromatography of samples and blanks with authentic standards. Quantities of metabolites were determined from the standard curves based on peak area (calculated automatically by a Hitachi 655-61 processor A). The standard curves were made by taking authentic standards through the same extraction procedures. The amounts of metabolites were expressed as nanomoles formed per gram of liver or per milligram of protein.

Results and Discussion

The metabolites produced by the incubations of 2,4-DNT and 2,6-DNT with microsomes from Wistar and

S.D. rat livers in the presence of NADPH were 2,4-DNB and 2,6-DNB, respectively. The rates of oxidation of 2,4-DNT and 2,6-DNT to 2,4-DNB and 2,6-DNB were linear with respect to the microsomal protein concentration (0 to 1 mg/ml), NADPH concentration (0 to 4 mM), incubation time (0 to 30 min) and substrate concentration (0 to 4 mM). As shown in Table I, the activity (about 14.8 nmol/g liver) for 2,4-DNT oxidation to 2,4-DNB in Wistar rat was about 3.1 times that (about 4.8 nmol/g liver) in S.D. rat. Similarly, the activity forming 2,6-DNB from 2,6-DNT in Wistar rat was higher than that in S.D. rat. In addition, the activity for 2,6-DNT oxidation to 2,6-DNB was higher than that for 2,4-DNT oxidation to 2,4-DNB in both strains.

The oxidation of 2,6-DNT to 2,6-DNB by the microsomal preparation containing NADPH from male Wistar rat livers was almost completely inhibited in an atmosphere of nitrogen (Fig. 1). In addition, this oxidation was inhibited by about 89% in an atmosphere of 80% CO in oxygen and inhibited by about 90% by a low concentration (0.1 mM) of SKF-525 A, an inhibitor of cytochrome P-450-dependent monooxygenase.⁶⁾ These findings indicate that the oxidation of 2,6-DNT to 2,6-DNB is mediated by the microsomal cytochrome P-450. The result in the micro-

TABLE I. Oxidation of 2,4-DNT and 2,6-DNT to Their Benzyl Alcohols by Hepatic Microsomal Preparations

Substrate	Strain	Incubation system		Activity (nmol of 2,4-DNB or 2,6-DNB formed/g liver)
		Enzyme source	Cofactor	
2,4-DNT	Wistar	MS	NADPH	14.77 $\pm$ 0.26
	S.D.	MS	NADPH	4.83 $\pm$ 1.34
2,6-DNT	Wistar	MS	NADPH	21.95 $\pm$ 2.53
	S.D.	MS	NADPH	13.44 $\pm$ 1.00

Incubation mixtures (2 ml) contained 65 mM phosphate buffer (pH 7.4), 4 mM NADPH, 1 mM substrate and 1.02 mg of microsomal protein. Incubations were carried out at 37°C for 20 min under air. Values are means $\pm$ S.D. for three samples. MS, microsomes.

Fig. 1. Effects of SKF-525 A, Co and N_2 on the Microsomal NADPH-Dependent Oxidation of 2,6-DNT to 2,6-DNB in Male Wistar Rat Livers

Incubation mixtures (2 ml) contained 65 mM phosphate buffer (pH 7.4), 1 mM NADPH, 0.25 mM 2,6-DNT, 1.34 mg of microsomal protein and 0 or 0.1 mM SKF-525 A. Incubations were carried out at 37°C for 15 min under air, 80% CO in oxygen or N_2 . Each bar represents the mean $\pm$ S.D. of three samples.

TABLE II. Oxidation of 2,4-DNB and 2,6-DNB to Their Benzaldehydes by Microsomal and Cytosol Preparations

Substrate	Strain	Incubation system		Activity (nmol of 2,4-DNBAl or 2,6-DNBAl formed/g liver)
		Enzyme source	Cofactor	
2,4-DNB	Wistar	MS	NADPH	0.47 ± 0.02
		CS	NAD	0.44 ± 0.01
	S.D.	MS	NADPH	0.33 ± 0.01
		CS	NAD	0.18 ± 0.01
2,6-DNB	Wistar	MS	NADPH	3.41 ± 0.25
		CS	NAD	1.26 ± 0.34
	S.D.	MS	NADPH	1.20 ± 0.04
		CS	NAD	0.44 ± 0.03

Incubation mixtures (2 ml) contained 65 mM phosphate buffer (pH 7.4), 4 mM cofactor, 1 mM substrate and 1.02 or 2.0 mg of microsomal or cytosol proteins. Incubations were carried out at 37 °C for 20 min under air. Values are means ± S.D. for three samples. CS, cytosol.

somal oxidation of 2,6-DNB to 2,6-DNBAl is in accord with the findings that 2,4-DNB oxidation to 2,4-DNBAl in male Fischer-344^{7,8)} and male S.D. rats¹⁾ is mediated by the microsomal cytochrome P-450.

Since the production of 2,4-DNBAl from 2,4-DNB in the fortified S.D. rat livers was shown to be mainly dependent on the microsomal preparation containing NADPH and cytosol preparation containing NAD,¹⁾ the metabolites produced by incubation of 2,4-DNB and 2,6-DNB with microsomal and cytosol preparations were examined by using NADPH and NAD, respectively, as cofactors. The metabolites produced by incubations of 2,4-DNB and 2,6-DNB with microsomal preparations containing NADPH were 2,4-DNBAl and 2,6-DNBAl in both strains. In addition, 2,4-DNBAl and 2,6-DNBAl from 2,4-DNB and 2,6-DNB were produced in the cytosol preparations containing NAD from livers of both strains. The productions of 2,4-DNBAl and 2,6-DNBAl were confirmed by co-chromatographies of the samples with authentic 2,4-DNBAl-Ph and 2,6-DNBAl-Ph after phenylhydrazine derivatization (chromatograms not shown). Table II shows the activities for production of 2,4-DNBAl and 2,6-DNBAl from 2,4-DNB and 2,6-DNB in both strains. The activities in the oxidations of the dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB) to dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) were very low compared with those for oxidations of the dinitrotoluenes (2,4-DNT and 2,6-DNT) to dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB) listed in Table I. However, the tendencies activity for production of 2,6-substituted dinitrobenzaldehyde (2,6-DNBAl) was higher than that for 2,4-substituted dinitrobenzaldehyde (2,4-DNBAl) in both strains, and that the activities for production of dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) in Wistar rat were higher than those in S. D. rat, are similar to those observed for formations of 2,4-DNB and 2,6-DNB from 2,4-DNT and 2,6-DNT in Wistar and S.D. rat livers (Table I). The oxidation of 2,6-DNB to 2,6-DNBAl by microsomal preparation containing NADPH from male Wistar rat livers was inhibited by about 49, 63 and 98% by addition of 0.1 mM SKF-525 A and in the atmospheres of 80% CO in oxygen, and nitrogen (Fig. 2).

In addition, the oxidation of 2,6-DNB to 2,6-DNBAl was

Fig. 2. Effects of SKF-525 A, CO and N₂ on the Microsomal NADPH-Dependent Oxidation of 2,6-DNB to 2,6-DNBAl in Male Wistar Rat Livers

Incubation mixtures (2 ml) contained 65 mM phosphate buffer (pH 7.4), 4 mM NADPH, 1 mM 2,6-DNB, 3.35 mg of microsomal protein and 0 or 0.1 mM SKF-525 A. Incubations were carried out at 37 °C for 20 min under air, 80% CO in oxygen or N₂. Each bar represents the mean ± S.D. of three samples.

Fig. 3. Oxidation of 2,6-DNB to 2,6-DNBAl by Yeast Alcohol Dehydrogenase Requiring NAD

Incubation mixtures (2 ml) contained 65 mM phosphate buffer (pH 7.4), 4 mM NAD, 1 mM 2,6-DNB, and 2 to 8 units of yeast alcohol dehydrogenase. Incubations were carried out at 37 °C for 20 min under air. Each point represents the mean ± S.D. of three samples.

mediated by yeast alcohol dehydrogenase, requiring NAD as a cofactor (Fig. 3). Those findings suggest that the NADPH-dependent microsomal oxidation of 2,6-DNB to 2,6-DNBAl is mediated by cytochrome P-450 and the NAD-dependent cytosolic oxidation is mediated by alcohol dehydrogenase. The result in the 2,6-DNB oxidation to 2,6-DNBAl is in accord with the finding¹⁾ that the 2,4-DNB oxidation to 2,4-DNBAl in male S.D. rat livers is mainly mediated by both microsomal cytochrome P-450 and cytosolic alcohol dehydrogenase. The participation of microsomal cytochrome P-450 in the oxidation of CH₂OH to CHO was reported by Watanabe *et al.*,⁹⁾ who found that the microsomal oxidation of 11-hydroxy- Δ^8 -tetrahydrocannabinol to 11-oxo- Δ^8 -tetrahydrocannabinol is mediated by a mixed function oxidase involving cytochrome P-450.

When dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) were incubated with microsomal and cytosol

TABLE III. Oxidation of 2,4-DNBAl and 2,6-DNBAl to Their Benzoic Acids by Microsomal and Cytosol Preparations

Substrate	Strain	Incubation system		Activity (nmol of 2,4-DNBAl or 2,6-DNBAl formed/g liver)
		Enzyme source	Cofactor	
2,4-DNBAl	Wistar	MS	NADP	20.90 ± 3.52
			NADH	0.82 ± 0.03
		CS	NAD	50.04 ± 3.14
	S.D.	MS	NADH	1.30 ± 0.10
			NADP	7.74 ± 1.60
		CS	NADH	0.20 ± 0.05
2,6-DNBAl	Wistar	MS	NAD	22.82 ± 0.82
			NADH	0.68 ± 0.10
		CS	NADP	n.d.
	S.D.	MS	NADH	n.d.
			NAD	1.28 ± 0.15
		CS	NADH	n.d.

Incubation mixtures (2 ml) contained 65 mM phosphate buffer (pH 7.4), 4 mM cofactor, 1 mM substrate and 1.02 or 2.0 mg of microsomal or cytosol proteins. Incubations were carried out at 37 °C for 20 min under air. Values are means ± S.D. for three samples. n.d., not detected.

TABLE IV. Reduction of 2,4-DNBAl and 2,6-DNBAl to Their Benzyl Alcohols by Microsomal and Cytosol Preparations

Substrate	Strain	Incubation system		Activity (nmol of 2,4-DNB or 2,6-DNB formed/g liver)
		Enzyme source	Cofactor	
2,4-DNBAl	Wistar	MS	NADP	4.99 ± 3.31
			NADH	152.60 ± 16.70
		CS	NAD	93.19 ± 1.00
	S.D.	MS	NADH	236.49 ± 4.77
			NADP	0.43 ± 0.01
		CS	NADH	60.70 ± 2.10
2,6-DNBAl	Wistar	MS	NAD	24.74 ± 2.97
			NADH	114.94 ± 4.27
		CS	NADP	1.28 ± 0.12
	S.D.	MS	NADH	39.88 ± 4.12
			NAD	12.11 ± 1.89
		CS	NADH	52.33 ± 6.12

Incubation mixtures (2 ml) contained 65 mM phosphate buffer (pH 7.4), 4 mM cofactor, 1 mM substrate and 1.02 or 2.0 mg of microsomal or cytosol proteins. Incubations were carried out at 37 °C for 20 min under air. Values are means ± S.D. for three samples.

preparations from livers of both strains, they were found to be metabolized to dinitrobenzoic acids (2,4-DNBAl and 2,6-DNBAl) and dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB). Since the dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) are produced by the oxidations of dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB) (Table II), this result indicates that the oxidations of the dinitrobenzyl alcohols to their benzaldehydes and the reductions of the benzaldehydes to their benzyl alcohols are reversible. Tables III and IV show the activities for oxidation of dinitrobenzaldehydes

Fig. 4. Effect of Hydralazine on the Cytosol NAD-Dependent Oxidation of 2,6-DNBAl to 2,6-DNBAl in Male Wistar Rat Livers

Incubation mixtures (2 ml) contained 65 mM phosphate buffer (pH 7.4), 2 mM NAD, 1 mM 2,6-DNBAl, 2.66 mg of cytosol protein, and 0 to 2 mM hydralazine. Incubations were carried out at 37 °C for 20 min under air. Each point represents the mean ± S.D. of three samples.

hydres (2,4-DNBAl and 2,6-DNBAl) to dinitrobenzoic acids (2,4-DNBAl and 2,6-DNBAl) and the activities for reduction of dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) to dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB), respectively. As shown in Table III, the activity in the oxidation of 2,4-DNBAl to 2,4-DNBAl is much higher than that in the oxidation of 2,6-DNBAl to 2,6-DNBAl in both strains.

The oxidation of 2,6-DNBAl to 2,6-DNBAl in cytosolic preparation containing NAD from male Wistar rat livers (Table IV) was inhibited by hydralazine (Fig. 4), an inhibitor of aldehyde oxidase.¹⁰⁾ This finding shows that the 2,6-DNBAl oxidation to 2,6-DNBAl is mediated by the cytosol aldehyde oxidase. The reduction of 2,6-DNBAl to 2,6-DNB in microsomal and cytosol preparations containing NADH from male Wistar rats livers (Table IV) was inhibited by 46 and 88%, respectively, by 1 mM *o*-phenanthroline, an inhibitor of aldehyde reductase¹¹⁾ (data not shown). Since Erwin *et al.*¹¹⁾ showed that an NADH-linked aldehyde reductase mediating the reduction of aliphatic and aromatic aldehydes such as heptaldehyde and *m*-nitrobenzaldehyde was inhibited about 60% by 5 mM *o*-phenanthroline, it seems that the microsomal and cytosolic reductions of 2,6-DNBAl to 2,6-DNB are mediated by the aldehyde reductases. The microsomal and cytosolic reductions of CHO to CH₂OH are also seen in the metabolism of 2,4-DNBAl in S.D. rat livers.¹⁾

Among the reactions examined, the activity for 2,4-DNBAl reduction to 2,4-DNB, which was mainly dependent on the microsomal preparation containing NADH and cytosol preparation containing NADH, was the highest in both strains (Table IV). The activity for reduction of 2,6-DNBAl to 2,6-DNB in both strains was also high compared with those of other reactions listed in Tables I, II and III. In conclusion, the present results that the activities for oxidation of dinitrotoluenes (2,4-DNT and 2,6-DNT) and dinitrobenzyl alcohols (2,4-DNB and 2,6-DNB) and for reduction of dinitrobenzaldehydes (2,4-DNBAl and 2,6-DNBAl) in Wistar rat livers are higher than those in S.D. rat livers (Tables I, II and IV), and that the activities for oxidation of benzaldehydes (2,4-DNBAl and 2,6-DNBAl) in Wistar rat livers are lower than those in S.D. rat livers

(Table III), indicate that there is a strain difference between Wistar and S.D. rats in the metabolism of DNT isomers.

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