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COVALENT INTERACTION OF 3,3′-DICHLOROBENZIDINE WITH HEPATIC LIPIDS

ENZYMIC BASIS AND STABILITY OF THE ADDUCTS

MICHAEL M. IBA,*† BEAT LANG,* PAUL E. THOMAS‡ and ANIMA GHOSAL*

Departments of *Pharmacology and Toxicology, and ‡Chemical Biology and Pharmacognosy, Rutgers University-Busch Campus, Piscataway, NJ 08854-0789, U.S.A.

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Abstract—Administration of a single oral dose (20 mg/kg) of [U-^{14}C]3,3′-dichlorobenzidine to rats resulted in the *in vivo* covalent binding of the compound to hepatic lipids. More than 70% of the lipid–3,3′-dichlorobenzidine adducts were accounted for in microsomes. Loss of the lipid-bound 3,3′-dichlorobenzidine residues from either total liver or endoplasmic reticulum occurred in at least two phases—an initial fast phase and a terminal slow phase. *In vitro* studies with hepatic microsomes in the presence of antibodies to specific P450 isozymes and chemical inhibitors to determine the enzymes that activate 3,3′-dichlorobenzidine to the lipid-binding derivative(s) implicated cytochrome P450d. The 3,3′-dichlorobenzidine-bound microsomal lipids were not mutagenic to Salmonella TA98 in the Ames test. The results suggest that adduct formation between 3,3′-dichlorobenzidine and membrane lipids may provide a measure of 3,3′-dichlorobenzidine activation. It is speculated that covalent interaction of the compound with membrane lipids may modify cellular processes, leading to either enhancement or attenuation of carcinogenesis by the chemical.

3,3′-Dichlorobenzidine (DCB),§ a precursor of commercial dyes and pigments, is carcinogenic in various tissues of many animal species and is a cancer suspect agent in humans [1]. Formation of the mutagenic and perhaps carcinogenic derivatives of the compound is catalyzed by liver microsomal P450 isozyme(s), one of which the compound itself is a good inducer [2, 3]. DCB also binds covalently to hepatic microsomal lipids in rats following either the *in vivo* administration of the compound or the *in vitro* addition to hepatic microsomal incubations [4, 5]. Consequences of the interaction of the compound with lipids in the rat include: diminution of vitamin E content of the endoplasmic reticulum *in vivo* [4], stimulation of the conjugation of the double bonds of unsaturated fatty acids *in vivo* and *in vitro* [5] and enhancement of NADPH-dependent peroxidation of the unsaturated fatty acids of liver microsomes from DCB-pretreated rats [4]. These changes suggest deleterious effects of the chemical at the membrane level, which may contribute to the carcinogenic effects of the chemical. Also, lipid adducts of other carcinogenic aryl amines have been reported to be mutagenic in the Ames test [6].

We reported previously that the activation of DCB to lipid-binding derivatives in hepatic microsomes was catalyzed by microsomal enzymes [5]. In the present studies, we investigated the microsomal enzymes involved in the reaction and assessed the mutagenicity of the lipid–DCB adducts. We also determined the kinetics of the DCB–lipid adducts formed *in vivo* as a means of assessing their stability. The results show that P450 isozyme d, the isozyme responsible for activating DCB to mutagenic derivatives [3], is involved also in the formation of the lipid-binding DCB derivatives. Loss of a fraction of the *in vivo*-derived DCB–lipid adducts from either total liver or the endoplasmic reticulum was very slow, suggesting persistence of some of the adducts. The potential of the lipid–DCB adducts to either enhance or attenuate carcinogensis by DCB is discussed in view of the established ability of altered membrane components to modify cellular functions.

METHODS

Materials. Male Sprague–Dawley rats (200–250 g body weight at the time of the experiment, from Taconic Farms, Germantown, NY) were acclimatized to, and maintained at, our animal facilities as described previously [5]. [U-^{14}C]DCB (sp. act. = 45.5 mCi/mmol; purity > 98%) was purchased from Pathfinders Laboratories (St. Louis, MO), and was purified further by HPLC prior to use. DCB·2HCl,

† To whom correspondence should be addressed at: Department of Pharmacology and Toxicology, Rutgers University-Busch Campus, P.O. Box 789, Piscataway, NJ 08854-0789.

§ Abbreviations: DCB, 3,3′-dichlorobenzidine; anti-P450 reductase (also anti-reductase), a polyclonal antibody raised in sheep against rat liver microsomal P450 reductase; anti-P450d(+c) (also anti-P450d), a polyclonal antibody raised in rabbits against rat liver microsomal P450d (P450IA2), which also cross-reacts with P450c (P450IA1) because of common epitopes; MAb, monoclonal antibody; IgG, immunoglobulin G; MAb C8, a monoclonal antibody raised in mice against rat liver microsomal P450c; MAb C7, a monoclonal antibody raised in mice against rat liver microsomal P450c but does not inhibit reactions catalyzed by the isozyme and, therefore, serves as a control for MAb C8; FMO, flavin-containing monooxygenase; TL, total hepatic lipids; ER, endoplasmic reticulum; ER:TL ratio, microsomal:total liver DCB-lipid adducts; NTU, α-naphthylthiourea.

NADP, NADPH, glucose-6-phosphate, methimazole and dilauroyl phosphatidylcholine were purchased from the Sigma Chemical Co. (St. Louis, MO). Glucose-6-phosphate dehydrogenase was purchased from Boehringer-Mannheim (Indianapolis, IN). α-Naphthylthiourea (NTU) was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Arachidonic acid, linoleic acid and oleic acid were purchased from NuCheck Prep (Elysian, MN). A monoclonal antibody (MAb) specific for P450c (P450IA2) (MAb C8), a polyclonal antibody made against P450d (P450IA2), which also recognizes P450c (anti-P450d(+c)), a polyclonal antibody against cytochrome P450 reductase (anti-P450 reductase), immunoglobulin G (IgG) from sheep or rabbit, and a monoclonal antibody specific for P450c (Mab C7), which serves as a control for MAb C8 because it does not inhibit any of the reactions catalyzed by P450c, were prepared as described previously [7]. Highly purified NADPH–cytochrome P450 reductase from rat liver was provided by Dr. G. S. Miwa, Merck, Sharp & Dohme, Inc. (Rahway, NJ).

Pretreatment of rats and preparation of subcellular fractions. For the determination of the kinetics of DCB–lipid adducts in the liver, sixteen rats were pretreated orally, each with 7.8 μmol [U-^{14}C]DCB (sp. act. = 3.2 mCi/mmol) in 0.2 ml Tween 80, and killed in groups of three or four after 6 hr, 9.5 hr, 14 hr, 24 hr, 48 hr, 96 hr, 8 days or 14 days. Total liver homogenates and microsomes from either control or pretreated rats were prepared by differential centrifugation as described previously [8]. Kinetic analysis of the disappearance of the *in vivo* DCB–lipid adducts was carried out using a non-linear computer program based on a two-compartment open model pharmacokinetic system.

For studies of the microsomal activation of DCB to lipid-binding derivatives, the rats were pretreated with DCB to induce cytochrome P450 as described previously [5], and microsomes were isolated by differential centrifugation [8].

For the isolation of total lipids, an aliquot of total liver homogenate or microsomal suspension in 0.1 M phosphate buffer, pH 7.4, was extracted twice, each with 2 vol. of ethyl acetate to remove any unreacted DCB and its metabolites. Total lipids in the resulting aqueous tissue precipitates were extracted twice, each with 2 vol. of chloroform : methanol (2 : 1), and quantitated gravimetrically as described previously [5]. DCB bound to the lipids was quantitated in the isolated lipid fractions by scintillation spectrometry as described previously [5].

Characterization of microsomal enzymes that catalyze DCB activation to lipid-binding derivatives. Liver microsomes from DCB-pretreated rats (1 mg protein) and 100 nmol [U-^{14}C]DCB (sp. act. = 3.7 mCi/mmol) were incubated with or without an NADPH-generating system [9] in the presence or absence of either α-naphthylthiourea (25 nmol) or methimazole (1 μmol), MAb C7 (0.25 mg protein) or MAb C8 (0.25 mg protein) or anti-P450d(+c) (6 mg protein) or rabbit IgG (6 mg protein), anti-P450 reductase (5 mg protein) or sheep IgG (5 mg protein). The ratio of each antibody protein to its enzyme protein antigen used is the ratio at which inhibition of catalytic activity of the enzyme is maximal [3, 7]. The reaction mixture (in a total vol. of 1 mL of 0.1 M phosphate buffer, pH 7.4) was incubated at 37° with shaking for 20 min under an atmosphere of either air, argon (100%) or carbon monoxide : oxygen (80 : 20) as described previously [5]. The assay was terminated by the addition of ethyl acetate followed by the extraction of lipids and quantitation of lipid-bound DCB as described above.

Assessment of DCB metabolism and activation by NADPH–cytochrome P450 reductase. The ability of NADPH–cytochrome P450 reductase to catalyze the activation of DCB to lipid-binding derivatives was assessed in a reconstituted system as follows: 1000 units of purified P450 reductase with or without 20 μg dilauryl phosphatidylcholine in 0.8 mL phosphate buffer (0.1 M, pH 7.4) was sonicated, followed by the addition of 100 nmol DCB (sp. act. = 3.7 mCi/mmol), and 1 mg of either arachidonic acid, linoleic acid or total hepatic microsomal lipids from untreated rats. The reaction mixture was made up to a total volume of 1 mL with phosphate buffer and the reaction initiated by the addition of 400 nmol NADPH (in 0.02 mL water). Incubation conditions and isolation of the lipids were as described above except that the initial ethyl acetate extracts were analyzed by HPLC for the presence of DCB metabolites, using a modification [10] of the procedure described previously [2]. An assay mixture to which no test lipids were added was also analyzed for the presence of DCB metabolites.

Assessment of the mutagenicity of DCB–lipid adducts. Lipids from liver microsomes either freshly isolated or previously incubated with [^{14}C]DCB and NADPH under air (as described above) were isolated as described above but under sterile conditions. The lipid extracts in chloroform, were dispensed in various aliquots, and the chloroform was evaporated under nitrogen. The lipid residues were then sonicated in 1 mL phosphate buffer (0.1 M, pH 7.4), and the resulting emulsion was assayed for mutagenicity in either the presence [11] or absence [12] of liver microsomal DCB-activating system.

RESULTS

Kinetics of DCB-binding lipids in the liver. Pretreatment of rats with DCB results in the covalent binding of the benzidine congener to hepatic microsomal lipids [5]. It was of interest to determine (i) the extent of the *in vivo* binding, (ii) the fractional contribution of the binding in microsomal lipids to total hepatic lipids, and (iii) the persistence in the endoplasmic reticulum and total liver of the lipid–DCB adducts. As shown in Fig. 1, DCB binding [expressed as pmol DCB bound/mg lipid (±SD of the mean)] at 6 hr, the earliest time-point examined following DCB administration, was 125 (±19) and 86 (±12) in total hepatic lipids (TL) and microsomes (ER) respectively. Thus, binding to the endoplasmic reticulum lipids accounted for 69% of the total initial (6 hr) binding to total hepatic lipids. At 14 hr, binding to total hepatic and endoplasmic reticulum lipids was 48 (±6) and 26 (±4), respectively, with the ratio of endoplasmic reticulum lipids : total hepatic lipids (ER : TL) being 0.53. At 14 days, TL and ER binding

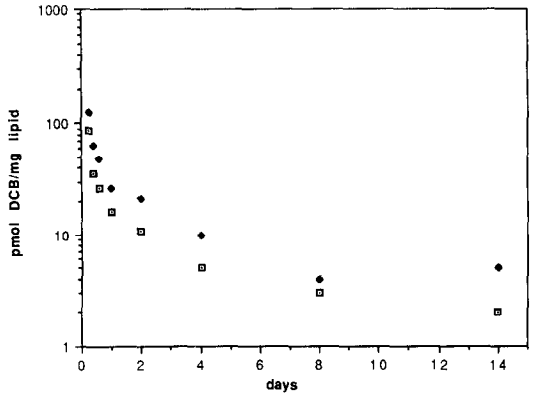

Fig. 1. Time course of *in vivo*-derived DCB–lipid adducts in total liver and microsomes. Each rat received 7.8 μmol [^{14}C-U]DCB (3.2 mCi/mmol, 20 mg/kg) orally, and the binding of DCB derivatives to lipids was determined as described in Methods. Each data point is the mean from a minimum of three rats. Numerical values (±SD) are given in the text. Closed symbols: total liver; open symbols: microsomes.

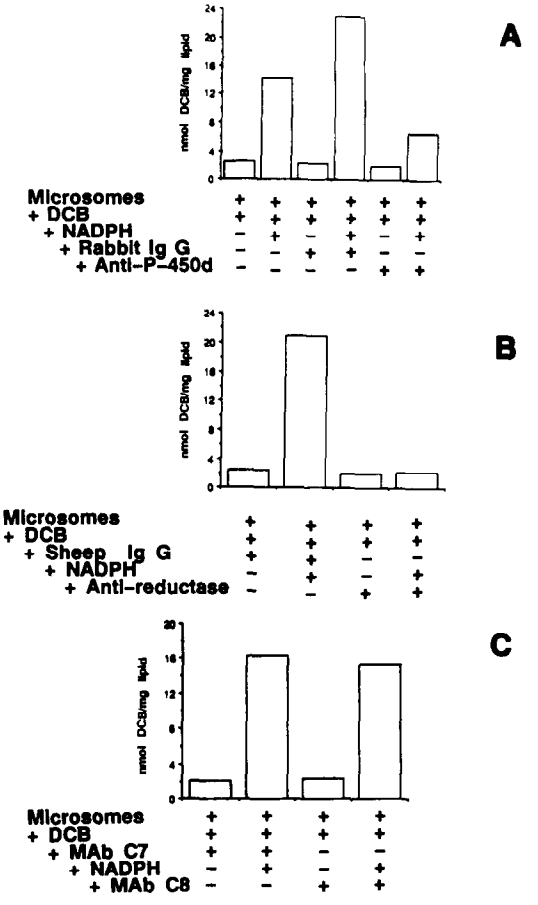

Fig. 2. Effect on the NADPH-dependent binding of [^{14}C]DCB to microsomal lipids of antibody to: cytochrome P450d (A), cytochrome P450 reductase (B), and cytochrome P450c (C). The assays were carried out as described in Methods. Binding (nmol DCB/mg lipid) in the absence and presence of NADPH was 2.4 and 14.2 respectively (panel A, first and second bars). In each of the panels, each value in the absence of NADPH has not been subtracted from the corresponding NADPH-dependent value. Each value is the average of two experiments.

was 5.3 (±2.6) and 2.5 (±18), respectively, with the ER:TL ratio being 0.4. Thus, the fraction of the binding contributed by the endoplasmic reticulum appeared to be maintained through most of the duration of the study.

The loss of DCB from either TL or ER lipids was biphasic. The disappearance half-lives of the rapid and slow phases were estimated to be 6.7 hr and 9.3 days, respectively, in the total hepatic lipids and 5.7 hr and 5.4 days, respectively in the ER fraction. The long terminal half-life (days) of the DCB-modified lipids contrasts with the very short half-lives (minutes to hours) reported for normal cellular membrane lipids, and suggests persistence of the DCB-modified lipids.

Effect of antibodies to cytochromes P450 and chemical inhibitors of microsomal enzymes on microsomal DCB activation to lipid-binding derivatives. The effects of chemical inhibitors suggested the involvement of P450 in the microsomal activation of DCB to lipid-binding derivatives [5]. To determine the microsomal P450 isozyme(s) involved, the assay was carried out in the presence of antibody to either cytochrome P450c or P450d, the isozymes induced in rat liver by DCB [3]. The microsomal monooxygenase, flavin-containing monooxygenase (FMO), has been implicated in the activation of DCB to mutagens [3]. Therefore, it was of interest to determine whether the enzyme also activated DCB to lipid-binding derivatives, using as probes α-naphthylthiourea and methimazole, both high affinity competitive inhibitors of the FMO [13, 14].

As shown in Fig. 2, the activation reaction was inhibited 78% by anti-P450d but was not affected by MAb C8, a monoclonal antibody to P450c. Doubling the amount of anti-P450d did not cause further inhibition of the reaction (data not presented). The results suggested that P450d but not P450c catalyzed the activation of DCB to lipid-binding derivatives. Each of the control immunoglobulins used in the study stimulated the activation of DCB to lipid-binding derivatives (Fig. 2), as it did the activation of DCB to mutagens [3]. The basis of the stimulation remains unknown.

The FMO inhibitors α-naphthylthiourea and methimazole did not inhibit the formation of lipid adducts (Table 1). Similarly, preincubation of microsomes for 3 min at 40°, which inactivates the FMO [13], did not prevent the binding (Table 1). These results suggested that DCB metabolites formed by the FMO did not bind to lipids. Carbon monoxide inhibited the reaction by 90% (Table 1), pointing to a predominant role of cytochromes P450 in the reaction. An anaerobic atmosphere (100% argon) did not affect the reaction significantly (Table 1), in agreement with the earlier (i) finding that the reaction did not require oxygen and, in fact, inhibited oxygen utilization, and (ii) speculation that formation of the lipid-binding DCB species may proceed by reductive mechanisms [5].

Table 1. Effect of chemical inhibitors on NADPH-dependent binding of [^{14}C]DCB to microsomal lipids

Incubation	DCB (nmol/mg lipid)
Microsomes/[^{14}C]DCB	2.4 ± 0.1
Microsomes/[^{14}C]DCB/NADPH	11.8 ± 1.5
Microsomes/40°/[^{14}C]DCB/NADPH	13.6 ± 2.8
Microsomes/[^{14}C]DCB/NADPH/NTU (25 μM)	12.0 ± 1.5
Microsomes/[^{14}C]DCB/NADPH/methimazole (1 mM)	10.8 ± 1.3
Microsomes/[^{14}C]DCB/NADPH/argon (100%)	9.7 ± 2.7
Microsomes/[^{14}C]DCB/NADPH/CO:O_2 (80:2)	1.1 ± 0.4*

The assays were performed as described in Methods except in the case of microsomes/40°/[^{14}C]DCB/NADPH, where microsomes were preincubated at 40° for 1 min prior to being used for the lipid binding assay. NTU = α-naphthylthiourea. Each value is the mean ± SD of three experiments, each carried out in duplicate. NADPH-independent binding (microsomes/[^{14}C]DCB) has been subtracted from each NADPH-dependent values. The NADPH-independent binding was not affected by any of the agents shown in the table.

* Significantly different from binding in the presence of NADPH alone (microsomes/[^{14}C]DCB/NADPH) ($P < 0.005$).

Role of cytochrome P450 reductase in the formation of lipid-binding DCB derivatives. Antibody to NADPH–cytochrome P450 reductase almost totally abolished the reaction (Fig. 2). The greater inhibition of the reaction by antibody to the reductase than by antibody to cytochrome P450d suggested at least two possibilities—involvement of P450 isozymes other than d and activation by the reductase *per se* of DCB to lipid-binding derivatives. The latter possibility was ruled out because in a reconstituted system containing the purified reductase and NADPH, with or without dilauroyl phosphatidylcholine, incubated in air or under nitrogen, DCB did not bind to added arachidonic acid, linoleic acid, oleic acid or total lipids extracted from rat liver homogenates or microsomes; under none of these conditions was a metabolite of DCB detected by HPLC analysis of the incubation mixture (data not presented).

Mutagenicity of DCB-lipid adducts to Salmonella TA98 in the Ames test. Lipid adducts formed by other arylamines, e.g. 2-aminofluorene, have been reported to be mutagenic in the Ames test to the frame-shift mutation sensitive Salmonella TA98. It has been speculated, therefore, that lipids may serve as reservoirs for the mutagenic derivatives of the compound [6]. Hence, it was of interest to determine whether the lipid adducts of DCB are mutagenic in the Ames test. The data in Table 2 show that the lipid–DCB adducts were devoid of mutagenicity to TA98 either directly or in the presence of an activating system. At equivalent concentrations, lipid-bound DCB was totally inactive compared to free DCB (Table 2).

DISCUSSION

An objective of the present study was to obtain information on the stability of the total DCB–lipid adducts formed in the liver *in vivo*. We showed previously that the binding of the chemical to lipids *in vivo* and *in vitro* is covalent and specific to only polyene-containing lipids [5]. In the present studies, we present evidence that disappearance of the DCB–lipid adducts from the liver is at least biphasic. The loss of radioactivity from the lipids most likely resulted from loss of entire lipid–DCB adducts rather than loss of the DCB moiety from the lipids. One rationale for this argument is the established rapid turnover of normal membrane lipids [15]. The different phases may represent the differential loss of the lipid-adducts from different subcellular fractions, classes of lipids or both. An outstanding feature of a fraction of the lipid adducts was its long half-life. This stability contrasts with the very short half-life of unmodified hepatic lipids, as determined by turnover studies [15]. The stability, perhaps, reflects the resistance of a pool of the adducted lipids to normal degradation processes.

The objective of the enzymic studies was to characterize the microsomal enzymes responsible for forming the lipid-binding DCB derivatives. Cytochrome P450d accounted for 78% of the activation. The reductase was essential for the reaction as evidenced by the 99% inhibition by antibody to the enzyme. However, participation of the reductase was not in the direct activation of DCB as evidenced by the absence of lipid binding by DCB and DCB metabolites in a reconstituted NADPH–cytochrome P450 reductase system. It is possible that all of the DCB activation was catalyzed by P450d. In that case, the anti-P450d-resistant activation (22% of the total activation) is explainable by inability of the antibody to totally inhibit P450d-catalyzed DCB activation. Alternatively, P450 isozymes other than d may have contributed the 22% of the activation.

The present studies also show that microsomal activation of DCB to lipid-binding derivatives is not catalyzed by the FMO. This contrasts with, the ability of the enzyme to catalyze the activation of DCB to mutagens [3]. P450d also catalyzes DCB activation to mutagens [3]. Possibly, at least two major classes of activated products are formed from DCB in microsomes: those mutagenic to TA98 and those that bind to lipids. The mutagens are most likely formed by

Table 2. Comparative mutagenicity of unmodified microsomal lipids, DCB-adducted microsomal lipids, and DCB

	TA98 Revertants/plate	
	A	B
Control lipids*		
1 mg	26 ± 3	29 ± 3
2 mg	27 ± 4	32 ± 3
DCB-adducted lipids†		
1 mg (13.8 nmol bound DCB residues)	30 ± 4	34 ± 5
2 mg (27.6 nmol bound DCB residues)	29 ± 5	32 ± 4
Methanol (10 μL)	32 ± 4	34 ± 3
DCB		
1 nmol	52 ± 5	81 ± 10
10 nmol	88 ± 10	612 ± 42
100 nmol	168 ± 12	1485 ± 118

Lipids were isolated from either freshly prepared microsomes from untreated rats (*) or microsomes preincubated with 100 nmol [^{14}C]DCB and NADPH (†) as described in Methods. Mutagenicity was assayed as described in the text either in the absence (A) or presence (B) of a rat liver microsomal activating system. DCB was added in 10 μL methanol. Each value is the mean ± SD of four determinations, each in triplicate.

P450d and the FMO, whereas the lipid-binding derivatives are likely to be formed predominantly by P450d at least in microsomes from DCB-pretreated rats.

Since formation of the lipid-binding DCB derivatives is catalyzed by microsomal enzymes, it was of interest to determine what fraction of the *in vivo* binding to total hepatic lipids is contributed by lipids of the endoplasmic reticulum. The results show that a majority (>70%) of the binding was contributed by microsomes, reflecting, perhaps, a major role of the endoplasmic reticulum in the *in vivo* activation of DCB as well as the presence in the organelle of target lipids. Interestingly, the kinetics of the loss of DCB from the lipids was the same in microsomes as in total liver and the fractional contribution of microsomes to total hepatic lipid binding was constant throughout the time course of the study (Fig. 1). The recovery of microsomes from total homogenate by differential centrifugation is not 100% [16]. Therefore, it is not unreasonable to speculate that the actual contribution of endoplasmic reticulum lipids to the total binding was much greater than the 70% observed, and that most of the radioactivity in the non-microsomal fraction may have represented unrecovered microsomes. Subcellular fractions other than the endoplasmic reticulum, whose lipid components activated DCB might bind to, are unknown at present, as are the exact chemical structures of the lipid adducts. However, the results of our electron paramagnetic resonance spectroscopic studies suggest that the lipid-binding DCB derivatives are most likely aryl radicals [17]. This contrasts with other carcinogenic arylamines whose lipid-binding derivatives have been characterized as the nitroso [6, 18], resulting from the oxidation of an N-hydroxy metabolite [19, 20]. The formation by DCB of predominantly aryl rather than nitroxyl adducts with lipids may also explain the lack of mutagenicity to Salmonella TA98 of the lipid–DCB adducts.

A pertinent question is the biological significance of the interaction of DCB with membrane lipids. One consequence, as reported previously [4, 5], is the diminution of vitamin E in the endoplasmic reticulum, with consequent enhancement of *in vitro* peroxidation of the isolated microsomes. However, neither *in vitro* nor *in vivo* is the binding of DCB to microsomal lipids attended by losses in cytochrome P450 (unpublished observation). The binding of the chemical to microsomal protein is low in comparison to lipids [5]. Other compounds, such as carbon tetrachloride, are metabolized to radical intermediates which bind to lipids and also undergo further metabolism oxidatively [21]. It is the oxidative metabolism of the compound that may be responsible for the binding to and destruction of proteins including cytochromes P450 [21]. Perhaps, the low binding of DCB to protein and the failure of the chemical to destroy P450 lie in the inhibition of oxygen uptake by microsomes during formation of the lipid-binding DCB species [5].

Regarding genotoxicity, it cannot be concluded at present that the binding of DCB to lipids is detoxifying since the genotoxicity test was carried out in only one test system. Studies are in progess to (i) establish the relationship, if any, of the TA98-positive DCB derivatives to the lipid-binding derivatives, and (ii) characterize the lipid adducts and assess their mutagenicity in test systems other than the Ames test.

Another consequence of the adduction of DCB to lipids is the poor recovery of DCB metabolites from both *in vivo* and *in vitro* DCB-metabolizing systems. Poor recovery of microsomal metabolites as a result of their binding to microsomal lipids has been reported for the tryptophan pyrolysate 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) [22].

Binding to lipids of activated arylamine derivatives may be assumed to lead to detoxification of the amine *per se*. However, the biological consequences of the adducted lipids, especially the persistent ones,

have not been studied. Several pieces of experimental evidence suggest that not all interactions between lipids and arylamines may be innocuous. The evidence includes the enhanced lipid peroxidation in hepatic microsomes from DCB-pretreated rats [4, 5], and the positive correlation between carcinogenicity and lipid binding by arylamines [18, 19] and nitrosamines [23].

We speculate further that whether or not the binding of DCB to lipids enhances or attenuates toxicity by the chemical is likely to depend on several factors. These may include the type of lipids adducted, their subcellular localization, and the biological functions served by the lipids in a cell. For example, in a cell, protein kinase C, whose activity is considered essential for mitogenesis in the progressional phase of chemical carcinogenesis [24], is activated by diacyl glycerol, a breakdown product of phospholipids, particularly phosphatidyl inositol [24]. Protein kinase C activity is modulated positively by phosphatidyl serine [24] but negatively by sphingolipids [25]. Mitogenesis is also dependent on Ca^{2+} release from the endoplasmic reticulum [24]. The latter process is mediated by inositol triphosphate (a product of membrane phosphatidyl inositol [24]) and depends on the structural integrity of the endoplasmic reticulum. Studies of the binding of DCB to the above-mentioned lipids, comparative formation of the lipid adducts in membranes of tissues susceptible and resistant to carcinogenesis by DCB, and the effects of the adducted lipids on cellular events related to mitogenesis, such as calcium homeostasis and protein kinase C activity, will further the understanding of the role of the lipid–DCB adducts in the carcinogenicity of the chemical.

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