Biochemical Pharmacology, Vol. 34, No. 17, pp. 3101–3106, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

FATTY ACID STIMULATED N-DEMETHYLATION OF 1,2-DIMETHYLHYDRAZINE AND TETRAMETHYLHYDRAZINE BY RAT COLONIC MUCOSA*

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(*Received* 9 *October* 1984; *accepted* 25 *January* 1985)

Abstract—A fatty acid stimulated, NADPH-independent pathway for the N-demethylation of 1,1-dimethylhydrazine (1,1-DMH) with the generation of HCHO was demonstrated in 10,000 *g* soluble fractions of colonic mucosal homogenates. Tetramethylhydrazine and, to a lesser extent, aminopyrine, but not 1,2-DMH or methylhydrazine, were also substrates for this reaction. Isolated superficial colonic epithelial cells metabolized 1,1-DMH at a faster rate than proliferative epithelial cells. Indomethacin, an inhibitor of cyclooxygenase activity, and 5,8,11,14-eicosatetraynoic acid (ETYA), an inhibitor of both cyclooxygenase and lipoxygenase activities, suppressed HCHO production from 1,1-DMH by 50 and 80%. However, in the presence of indomethacin or ETYA, arachidonate hydroperoxide stimulated HCHO formation. This suggested a peroxidative mechanism for 1,1-DMH metabolism, related in part to prostaglandin synthesis. A possible role for lipoxygenase activity in mediating 1,1-DMH metabolism was suggested by the ability of linoleate, which did not increase prostaglandin synthesis, to stimulate 1,1-DMH metabolism and by the fact that ETYA was more effective than indomethacin as an inhibitor of 1,1-DMH metabolism. The fatty acid stimulated pathway for N-demethylation was clearly distinct from the mixed function oxidase activities. NADPH did not stimulate 1,1-DMH metabolism to HCHO. 7,8-Benzoflavone or SKF-525A, inhibitors of cytochrome P-450, and methimazole, an inhibitor of N-demethylation catalyzed by the hepatic microsomal FAD-containing monooxygenase, did not suppress HCHO formation. To the extent that 1,1-DMH and tetramethylhydrazine reach the colon unchanged, the results suggest that fatty acid stimulated cooxidation pathways in colonic mucosa may contribute to the metabolism of these agents. Metabolism by superficial cells which are destined to slough may be an important defense mechanism against the toxic and carcinogenic actions of these hydrazines in colon.

Previous studies with several tissues including ram seminal vesicles, renal inner medulla and colonic mucosa [1–8] have demonstrated the cooxidative metabolism of several classes of drugs and carcinogens by the prostaglandin synthase complex. This pathway may be particularly important in the local metabolism of xenobiotics by tissues, such as colon, with low cytochrome P-450 activity [9, 10]. Recently, the N-dealkylation of several monomethyl and dimethyl substituted anilines [11], aminopyrine [12] and tetramethylhydrazine [13] by the prostaglandin synthase activity of ram seminal vesicle microsomes has been demonstrated.

In the present study, we examined and compared the ability of 10,000 *g* soluble fractions of colonic mucosal scrapings or epithelial cell homogenates to metabolize a series of alkyl hydrazines via the fatty acid stimulated pathway. The results demonstrate that fatty acids stimulated the N-demethylation of 1,1-dimethylhydrazine (1,1-DMH)‡ and tetramethylhydrazine by a mechanism that is distinct from the NADPH-dependent mixed function oxidase systems and is linked, at least in part, to prostaglandin synthesis is colon mucosa. Evidence for the involvement of lipoxygenase activity in the N-demethylation of 1,1-DMH and tetramethylhydrazine is also presented. Of note, 1,2-DMH and methylhydrazine do not appear to be substrates for this pathway.

* Supported by Grant CA 31680 from the National Cancer Institute through the Large Bowel Cancer Project.

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‡ Abbreviations: DMH, dimethylhydrazine; ETYA, 5,8,11,14-eicosatetraynoic acid; SKF-525A, 2-diethylaminoethyl-2,2-diphenylvalverate; and PG, prostaglandin.

METHODS

Tissue preparation. Female Sprague–Dawley rats (Zivic–Miller Laboratories, Pittsburgh, PA) were anesthetized with pentobarbital (5 mg/100 g, i.p.), and the distal colon was resected from the colonic flexure to 1 cm above the anal orifice and placed in 0.85% NaCl at 4° which contained 30 mg penicillin and 5 mg gentamicin per 100 ml. The colons were cleaned and opened longitudinally, and mucosal scrapings were prepared with a metal spatula. Alternatively, superficial and proliferative colonic epithelial cells were prepared as previously described [14, 15]. Approximately 90–95% of the isolated cells were epithelial cells as determined by light microscopy. A section of residual colon was also routinely examined histologically to ensure complete removal of epithelium. Greater than 95% of the epithelial cells were viable as determined by trypan blue exclusion. Thorough washing of cells was necessary to remove dithiothreitol, which was found to suppress colonic prostaglandin synthesis.

As previously reported [14, 15], the *in vivo* rate of [^{3}H]dThd incorporation into DNA of cells from normal adult rat colon isolated in fraction V is 5- to 10-fold higher than that in fraction I. The rate of [^{3}H]dThd incorporation into DNA of cells from rat colon in pooled fractions IV and V is 4- to 7-fold higher than that in pooled fractions I and II. The *in vivo* rate of [^{3}H]dThd incorporation into DNA was determined in epithelial cells that had been isolated from the colons of rats killed 2 hr after injection of 50 μCi of [^{3}H]dThd, as previously described in detail [14, 15]. Pooled fractions IV and V contained approximately 65–68% of the total protein and DNA recovered in fractions I through V, whereas pooled fractions I and II contained 17–25% of the total recovered protein and DNA [14, 15].

Mucosal scrapings (100 mg tissue/ml buffer) or epithelial cells (100 mg wet wt of cells/1 ml buffer) were homogenized in 50 mM Tris, 2 mM EDTA, pH 7.4. Homogenates were centrifuged at 10,000 *g*. The freshly prepared 10,000 *g* supernatant fraction was incubated for various periods of time in a final volume of 0.5 ml with test agents present at the final concentration indicated in the text.

Determination of HCHO. For determination of HCHO, incubations were stopped by addition of 0.25 ml of cold 20% trichloroacetic acid. Following centrifugation, 0.5 ml of the clear supernatant fraction was assayed for HCHO by the procedure of Nash [16]. Standard curves were linear between 7 and 140 nmoles HCHO. When tested at 5 mM, 1,1-DMH and methylhydrazine were found to suppress color development in the HCHO assay by 53 and 50% respectively. The results shown were corrected accordingly. 1,2-DMH, tetramethylhydrazine or aminopyrine did not influence the standard curve for HCHO when tested at 5 mM. The generation of HCHO from 1,1-DMH and tetramethylhydrazine was linear with time between 15 and 60 min and with protein between 1 and 3 mg.

Determination of hydrazine concentration. For determination of hydrazine concentration by the method of Watt and Chrisp [17], reactions were stopped by the addition of an equal volume of 2 N HCl and centrifuged at 3000 *g*. A 0.8-ml aliquot of the extract was mixed with the *p*-dimethylaminobenzaldehyde color reagent, the mixture was incubated for 10 min at ambient temperature, and absorbance was measured at 458 nm. Standard curves were linear from 0.5 to 8 nmoles methylhydrazine. The assay does not distinguish between hydrazine and methylhydrazine. Reaction of 50 mM 1,1-DMH with *p*-dimethylaminobenzaldehyde in the absence of the colonic mucosal 10,000 *g* supernatant fraction did not produce sufficient absorbance at 458 nm to contribute significantly to that generated by reaction of 1,1-DMH with the color reagent in the presence of the colonic mucosal soluble fraction.

Preparation of arachidonate hydroperoxide. 15-Hydroperoxy-5,8,11,13-eicosatetraenoic acid was prepared by incubation of arachidonate with soybean lipoxygenase in 50 mM borate buffer at pH 9.0 for 5 min at 0° as previously described [18]. Protein was determined by the method of Lowry *et al.* [19]. Statistical significance was determined by Student's independent *t*-test. Experiments were repeated three or four times, and the average value of duplicate determinations from each experiment was entered as a single number (df = 4 or 6, respectively, comparing any two variables by the independent *t*-test).

Materials. 5,8,11,14-Eicosatetraynoic acid (ETYA) and SKF-525A were gifts of Hoffmann-LaRoche, Inc., Nutley, NJ, and Smith Kline & French Laboratories, Philadelphia, PA, respectively. Soybean lipoxygenase (Type I), methimazole and 1,2-DMH were obtained from the Sigma Chemical Co., St. Louis, MO. 1,1-DMH was purchased from the Aldrich Chemical Co., Milwaukee, WI. Tetramethylhydrazine and methylhydrazine were obtained from the Fluka Chemical Co., New York, NY. The sources of all other reagents have been described previously [8, 14, 15].

RESULTS

Figure 1 illustrates the metabolism of 1,1-DMH to HCHO by the 10,000 *g* soluble fraction of colonic mucosal homogenates in the presence of arachidonate. HCHO formation increased with 1,1-DMH concentration between 1 and 50 mM, with half-maximal HCHO production occurring at 5 mM 1,1-DMH. In separate experiments (not shown), methylhydrazine or hydrazine production was measured in 60-min incubations of the 10,000 *g* soluble fraction of colonic mucosa with 50 mM 1,1-DMH and found to be 70% of the value obtained for HCHO. HCHO production from 1,1-DMH was linear with time for 15–60 min and with protein concentration between 1 and 3 mg. In separate experiments, optimal production of HCHO from 1,1-DMH occurred at pH 7.5 to 8 (not shown). Similar results were obtained when tetramethylhydrazine was employed as substrate. By contrast, aminopyrine was much less efficiently metabolized to HCHO by the colonic mucosal

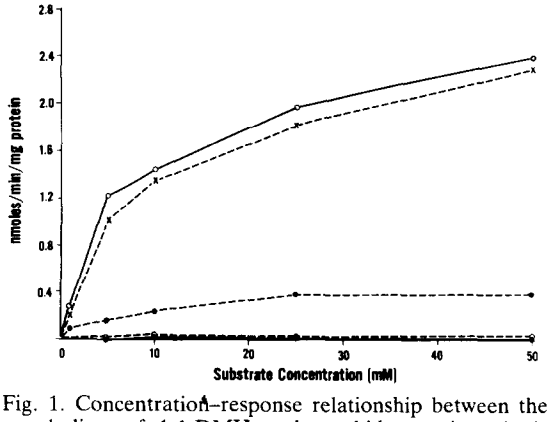

Fig. 1. Concentration–response relationship between the metabolism of 1,1-DMH and arachidonate in colonic mucosal homogenates. The 10,000 *g* soluble fraction of colonic mucosal scraping homogenates was incubated for 60 min at 37° with 1,1-dimethylhydrazine (○——○), tetramethylhydrazine (×– – –×), aminopyrine (●– – –●), 1,2-dimethylhydrazine (○– – –○) or monomethylhydrazine (●——●) at the indicated final concentration. All incubates contained 100 μM arachidonate. At the end of 30 min, HCHO content was extracted and assayed as described in Methods. Results shown are means ± S.E. of duplicate determinations from a single experiment verified in two additional separate studies.

Table 1. Effects of indomethacin and ETYA on basal and fatty acid stimulated production of HCHO from 1,1-dimethylhydrazine in 10,000 *g* soluble fraction of colonic mucosa*

	HCHO (nmoles/min/mg protein)				
	Final additions				
Initial additions	None	Arachidonate	Linoleate	Palmitate	Arachidonate hydroperoxide
None	0.78 ± 0.16	1.2 ± 0.2†	1.2 ± 0.1†	0.72 ± 0.13	1.3 ± 0.2†
Indomethacin	0.37 ± 0.05‡	0.73 ± 0.08‡	1.3 ± 0.2†	0.33 ± 0.05‡	1.2 ± 0.1†
ETYA	0.16 ± 0.02‡	0.12 ± 0.02‡	0.15 ± 0.02‡	0.12 ± 0.02‡	1.3 ± 0.2†

* The 10,000 *g* soluble fraction of colonic mucosal scraping homogenates was preincubated for 10 min at 37° with no addition, indomethacin (300 μM) or ETYA (500 μM) as indicated under initial additions. 1,1-Dimethylhydrazine (5 mM) was then added, followed by arachidonate (100 μM), linoleate (100 μM), palmitate (100 μM) or arachidonate hydroperoxide (25 μM), and the incubation was continued for an additional 60 min. At the end of 60 min, HCHO was extracted and assayed as described in Methods. Results shown are means ± S.E. of eight determinations from four separate experiments.

† $P < 0.05$, compared to corresponding value in the absence of a final addition.

‡ $P < 0.05$, compared to corresponding value in the absence of an initial addition.

10,000 *g* supernatant fractions than either 1,1-DMH or tetramethylhydrazine, and no significant HCHO production was observed with either 1,2-DMH or methylhydrazine (Fig. 1).

The metabolism of 1,1-DMH, tetramethylhydrazine and aminopyrine to HCHO in Fig. 1 was assessed in the presence of 100 μM arachidonate. As shown in Table 1 for 1,1-DMH, significant HCHO production was observed in the absence of exogenous arachidonate. Addition of arachidonate, linoleate or arachidonate hydroperoxide increased HCHO production approximately 50%. By contrast, the saturated fatty acid palmitate was without effect. HCHO production from 1,1-DMH was dependent on the presence of the colonic mucosal 10,000 *g* soluble fraction. No effects of fatty acids or fatty acid hydroperoxide were observed in the absence of tissue supernatant fraction. As is also shown in Table 1, the cyclooxygenase inhibitor indomethacin (300 μM) suppressed HCHO production approximately 50–60% in the presence or absence of arachidonate. By contrast, indomethacin had no effect on linoleate- or arachidonate-hydroperoxide-induced increases in HCHO production. ETYA (500 μM), an inhibitor of cyclooxygenase and lipoxygenase activities in some tissues [20], suppressed HCHO production by 80% in the absence of fatty acids and abolished the stimulatory effects of arachidonate and linoleate on 1,1-DMH metabolism. The suppressive effects of ETYA were overcome by exogenous arachidonate hydroperoxide (Table 1).

Table 2 illustrates the concentration–response relationship between indomethacin or ETYA and the generation of HCHO from 1,1-DMH in the 10,000 *g* soluble fraction of colonic mucosal scraping homogenates. As shown, indomethacin at 25 μM reduced basal and arachidonate-stimulated HCHO formation by approximately 30%. No significant suppression was detected with 10 μM indomethacin (not shown). Indomethacin at 100 μM further suppressed basal and arachidonate-stimulated increases in HCHO formation by about 50% but did not abolish the stimulatory effect of arachidonate on HCHO formation. Increasing the concentration of indomethacin to 300 μM did not further suppress HCHO generation in the presence or absence of arachidonate. ETYA at 25 μM suppressed basal and arachidonate-stimulated HCHO formation by 45–50%. No significant suppression was detected with 10 μM ETYA (not shown). Increasing the ETYA concentration to 100 μM further suppressed basal and arachidonate-stimulated HCHO formation by 75–

Table 2. Concentration–response relationship between indomethacin or ETYA and the generation of HCHO from 1,1-DMH in 10,000 *g* soluble fraction of colonic mucosa*

		HCHO (nmoles/min/mg protein)	
		Final additions	
Initial additions		None	Arachidonat
None		0.67 ± 0.08	1.1 ± 0.1†
Indomethacin	25 μM	0.48 ± 0.06‡	0.78 ± 0.09†‡
	100 μM	0.33 ± 0.05§	0.55 ± 0.08†§
	300 μM	0.29 ± 0.04§	0.52 ± 0.07†§
ETYA	25 μM	0.32 ± 0.04‡	0.61 ± 0.08†‡
	100 μM	0.15 ± 0.03§	0.28 ± 0.04†§
	500 μM	0.13 ± 0.01§	0.15 ± 0.02†§

* The 10,000 *g* soluble fraction of colonic mucosal scraping homogenates was preincubated for 10 min at 37° with no addition or the indicated concentrations of indomethacin or ETYA. 1,1-Dimethylhydrazine (5 mM) was then added to all the incubations. Arachidonate (100 μM) was added where indicated, and the incubation was continued for 60 min. Results shown are means ± S.E. of six determinations from three separate experiments.

† $P < 0.05$, compared to corresponding value in the absence of arachidonate.

‡ $P < 0.05$, compared to corresponding value in the absence of an initial addition.

§ $P < 0.05$, compared to corresponding value obtained with 25 μM indomethacin or ETYA.

Table 3. Effects of indomethacin and ETYA on basal and arachidonate-stimulated HCHO production from 1,1-dimethylhydrazine in superficial versus proliferative epithelial cell homogenates*

	HCHO (nmoles/min/mg protein)	
	Arachidonate	
	−	+
Superficial cell homogenate	0.9 ± 0.1	1.4 ± 0.2†
+ Indomethacin	0.48 ± 0.06‡	0.92 ± 0.1†‡
+ ETYA	0.14 ± 0.02‡	0.26 ± 0.03†‡
Proliferative cell homogenate	0.24 ± 0.03§	0.38 ± 0.04†§
+ Indomethacin	0.11 ± 0.01‡§	0.16 ± 0.01†‡§
+ ETYA	0.037 ± 0.005‡§	0.054 ± 0.007†‡§

* The 10,000 *g* soluble fraction of superficial and proliferative epithelial cell homogenates was preincubated for 10 min at 37° in the presence or absence of indomethacin (100 μM) or ETYA (100 μM) as indicated under preincubated conditions. 1,1-Dimethylhydrazine (5 mM) was then added to all the incubates. Arachidonate (100 μM) was then added to some of the incubates as indicated, and the incubation was continued for an additional 60 min. At the end of the incubation, HCHO was extracted and assayed as described in Methods. Results shown are means ± S.E. of six determinations from three separate experiments.

† $P < 0.05$, compared to corresponding value in the absence of arachidonate.

‡ $P < 0.05$, compared to corresponding value in the absence of indomethacin or ETYA.

§ $P < 0.05$, compared to corresponding value in the superficial cell homogenate.

80% of the value observed in the absence of ETYA but did not abolish the stimulatory effect of arachidonate on this variable. Increasing ETYA to 500 μM did not further suppress basal HCHO formation, but abolished the stimulatory effect of arachidonate on this variable.

Our own previous studies have shown that prostaglandin production is 3–4 times higher in superficial colonic epithelial cells compared to proliferative cells [14]. As illustrated in Table 3, 10,000 *g* soluble fractions superficial cell homogenates were 3.5–4 times more active at metabolizing 1,1-DMH to HCHO than that from proliferative cells, whether incubations were conducted in the presence or absence of arachidonate. Addition of indomethacin (100 μM) suppressed 1,1-DMH metabolism by about 35–55% in the presence or absence of arachidonate. ETYA (100 μM) suppressed HCHO production from 1,1-DMH by 80–85% in both superficial and proliferative epithelial cell homogenates in the presence or absence of arachidonate.

As illustrated in Table 4, addition of NADPH

Table 4. Effects of NADPH and microsomal mixed function oxidase inhibitors on production of HCHO from 1,1-dimethylhydrazine*

	HCHO (nmoles/min/mg protein)	
	Final additions	
Initial additions	None	NADPH + $MgCl_2$
None	0.79 ± 0.14	0.72 ± 0.09
7,8-Benzoflavone	0.76 ± 0.13	0.73 ± 0.14
SKF-525A	0.72 ± 0.09	0.78 ± 0.08
Methimazole	0.74 ± 0.11	0.71 ± 0.12

* The 10,000 *g* soluble fraction of colonic mucosal scraping homogenates was preincubated for 10 min at 37° with 7,8-benzoflavone (0.5 mM), SKF-525A (0.5 mM) or methimazole (0.5 mM) as indicated under initial additions. 1,1-Dimethylhydrazine (5 mM) was then added to all the incubates. NADPH (0.5 mM) plus $MgCl_2$ (5 mM) was then added where indicated under final additions, and the incubation was continued for an additional 60 min. At the end of 60 min, HCHO content was extracted and assayed as described in Methods. Results shown are means ± S.E. of six determinations from three separate experiments.

plus $MgCl_2$ to the 10,000 *g* soluble fraction did not influence the generation of HCHO from 1,1-DMH. Moreover, 7,8-benzoflavone and SKF-525A, inhibitors of colonic mucosal cytochrome P-450 activity [9, 10] did not suppress 1,1-DMH metabolism in the presence or absence of NADPH. The predominant pathway for 1,1-DMH metabolism in the liver is NADPH-dependent, microsomal, FAD-containing monooxygenase activity [21]. However, as shown in Table 4, methimazole, an inhibitor of hepatic microsomal monooxygenase [21], was also without effect on 1,1-DMH metabolism in the 10,000 *g* soluble fraction of colonic mucosal scrapings.

DISCUSSION

In the present study, the metabolism of 1,1-DMH and tetramethylhydrazine by a fatty acid stimulated pathway, which is at least in part linked to cyclooxygenase activity, was observed in 10,000 *g* soluble fractions of colonic mucosa. Aminopyrine, which was demonstrated previously to be oxidized by prostaglandin synthase of ram seminal vesicle microsomes [12], was a relatively poor substrate for the colonic mucosal soluble fraction, compared to either 1,1-DMH or tetramethylhydrazine. Moreover, 1,2-DMH and methylhydrazine did not appear to be substrates for the fatty acid stimulated pathway. The results suggest substrate preference for 1,1-di-substituted hydrazines. Nevertheless, they must be interpreted with caution. Only a limited number of alkyl hydrazines were tested. Moreover, with respect to methylhydrazine, it is possible that the reactivity of this compound could result in (a) a reduction in substrate concentration below that needed to see significant methylhydrazine metabolism, or (b) formation of moieties which could cause enzyme inactivation. Either of these effects, rather than a substrate specificity, could have contributed to our failure to see significant HCHO production from methylhydrazine.

In the present study, 1,1-DMH was shown to be metabolized to HCHO in the absence of added nucleotide or fatty acid co-factors. Addition of arachidonate to the incubations increased N-demethylation. A link between oxygenation of arachidonate by cyclooxygenase and N-demethylation of 1,1-DMH and tetramethylhydrazine was supported by the ability of indomethacin to suppress basal and arachidonate-stimulated N-demethylation. However, involvement of colonic lipoxygenase activity in this reaction was implied by (a) the ability of linoleic acid, which is not a substrate for prostaglandin synthesis, to stimulate N-demethylation of 1,1-DMH and tetramethyldrazine, and (b) greater suppression of basal and arachidonate- or linoleate-induced stimulation of this variable by ETYA than by indomethacin. Thus, ETYA, a known inhibitor of both cyclooxygenase and lipoxygenase activities [20], was more effective than indomethacin in the suppression of the N-demethylation reaction, consistent with involvement of both the cyclooxygenase and lipoxygenase pathway in this fatty acid dependent process. Similar findings were observed in our previous study of fatty acid stimulated benzo[*a*]-pyrene metabolism by colonic mucosa [8].

Several lines of evidence indicated that the fatty acid stimulated pathway for N-demethylation of 1,1-DMH and tetramethyldrazine was distinct from the cytochrome P-450- and FAD-dependent monooxygenase pathways. Fatty acid stimulated N-demethylation did not require NADPH and was not suppressed by SKF-525A or 7,8-benzoflavone, inhibitors of microsomal cytochrome P-450 in colon [9, 10], or by methimazole, an inhibitor of hepatic microsomal FAD-dependent monooxygenase activity [21]. Moreover, the substrate specificity for fatty acid stimulated N-demethylation differed from that reported for both the cytochrome P-450 and monooxygenase activities of hepatic microsomes. Hepatic monooxygenase activity containing FAD metabolizes both 1,2- and 1,1-DMH [21], while the cytochrome P-450 system metabolizes 1,2- but not 1,1-DMH [22]. By contrast, the fatty acid stimulated pathway metabolizes 1,1- but not 1,2-DMH. In the case of 1,1-DMH, the pH optimum for the production of HCHO by the fatty acid stimulated system in colonic mucosa clearly differed from that reported for the hepatic FAD-dependent monooxygenase [21] (pH 7.5 versus 8.4 respectively). To the extent that the properties of microsomal cytochrome P-450 and FAD-dependent monooxygenase are similar in liver and colon mucosa, these observations support the existence of a distinct fatty acid stimulated metabolic pathway for N-demethylation of 1,1-DMH and tetramethylhydrazine in colonic mucosa.

When administered systemically, 1,2-DMH is a potent and relatively specific carcinogen for the colon [22]. By contrast, 1,1-DMH and tetramethylhydrazine induce tumors in blood vessels and lung but are only weakly carcinogenic for colon [23, 24]. The biologic significance of the ability of the colonic mucosa to metabolize 1,1-DMH and tetramethylhydrazine to HCHO is not known. The ultimate carcinogenic forms of these agents and the role of metabolism in their activation and/or detoxification are not well understood. However, in this regard, metabolism of 1,2-DMH to HCHO plus methylhydrazine has been proposed previously as a detoxification pathway. Since methylhydrazine and hydrazine are at best only weak colon carcinogens [23, 25, 26], the finding in the present study that methylhydrazine and/or hydrazine are generated during 1,1-DMH metabolism supports a role for fatty acid dependent metabolism of 1,1-DMH in the detoxification of this agent. Whether the failure of 1,2-DMH to serve as a substrate for fatty acid stimulated metabolism in colon, and thereby be inactivated by this pathway, contributes to its carcinogenicity in colon is not known. However, initial metabolism of 1,2-DMH to methylazoxymethanol in liver is thought to be an essential determinant of its carcinogenicity in colon [27–30]. Accordingly, colonic metabolism of methylazoxymethanol and not 1,2-DMH is likely the salient issue with respect to expression of the carcinogenic activity of 1,2-DMH [27–30]. Finally, the observation in the present study that superficial colonic epithelial cells, which are destined to slough, were three times more active in the metabolism of 1,1-DMH and tetramethylhydrazine than the proliferative cells is also consistent with a role for fatty acid stimulated N-demethylation

as a potential mechanism for the protection of colon against expression of any toxic and/or carcinogenic effects of these agents on colon. Clearly, *in vivo* studies are needed to determine the significance of the fatty acid stimulated N-demethylation of some alkyl hydrazines in the expression of their carcinogenic actions.

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