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IN VIVO PRODUCTION OF ETHYLENE FROM 2-KETO-4-METHYLTHIOBUTYRATE IN MICE

GLEN D. LAWRENCE* and GERALD COHEN
Department of Neurology and Alcohol Research Center, Mount Sinai School of Medicine, New York, NY 10029, U.S.A.

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Abstract—The use of 2-keto-4-methylthiobutyric acid (KMB), the alpha-keto analog of methionine, was studied as a potential means of detecting free radical generation *in vivo*. KMB-dependent ethylene production (presumably from free radical interception), and ethane production from *in vivo* lipid peroxidation, were monitored simultaneously by measuring the rate of exhalation of these hydrocarbons by mice. Injection of KMB (1 g/kg) into mice resulted in an 8-fold increase in ethylene production above endogenous levels seen in saline-injected controls (1.47 ± 0.35 vs 0.17 ± 0.02 nmoles/100 g/hr respectively). Administration of CCl_4 (3.0 g/kg) to initiate hepatic lipid peroxidation, 20 min prior to KMB injection, augmented the production of ethylene (2.37 ± 0.10 nmoles/100 g/hr). Lipid peroxidation following injection of CCl_4 was monitored via the increased exhalation of ethane. Pretreating the mice with vitamin E (100 mg/kg daily for 3 days), an inhibitor of lipid peroxidation, did not result in a significant change in ethylene production from KMB by itself or after prior injection of CCl_4. However, vitamin E did suppress ethane production initiated by CCl_4. Similar results were obtained with mouse liver slices studied *in vitro*. Metyrapone (150 mg/kg), an inhibitor of hepatic mixed function oxidase activity, also suppressed significantly the CCl_4-stimulated production of ethane, but not the CCl_4-stimulated production of ethylene from KMB. It appears that ethylene production from KMB does not derive from free radicals generated during *in vivo* lipid peroxidation since suppression of lipid peroxidation by vitamin E or metyrapone did not suppress ethylene production.

Free radicals, and oxy-radicals in particular, have received widespread attention in recent years as intermediates or agents in drug metabolism, lipid peroxidation, tissue pathology, carcinogenesis and aging. Free radicals, and oxy-radicals especially, tend to be highly reactive and seldom achieve sufficient concentration in a viable cell to be detected by direct methods, such as electron spin resonance spectroscopy, although stable radical adducts with spin-trapping agents have been measured *in vitro* (e.g. see Refs. 1 and 2). The predominant method of detecting free radical involvement in a biochemical process is through the use of a radical scavenger and subsequent measurement of products of the interaction of the scavenger with a free radical. One criterion for the use of a scavenger *in vivo* is that a readily detectable product must be formed. Methional and 2-keto-4-methylthiobutyrate (KMB) act as radical scavengers [3–9] and produce ethylene as a readily detectable gaseous product. Compared to other thioethers, KMB and methional are the most active in forming ethylene from radical-mediated processes [10]. KMB and methional scavenge hydroxyl radicals and alkoxyl radicals [11–13]. The excitation of flavin mononucleotide by light in a nitrogen atmosphere also generates ethylene from KMB [10].

This study was undertaken to determine whether the production of ethylene from KMB or methional could be detected *in vivo*. Ethylene is exhaled and can be measured by methods which were developed previously for measuring ethane production from *in vivo* lipid peroxidation [14]. Carbon tetrachloride was used to induce *in vivo* hepatic lipid peroxidation in mice; alkyl, alkoxyl and peroxyl radicals are intermediates in lipid peroxidation. Ethylene production from KMB, with stimulation by CCl_4, was observed *in vivo*. However, experiments with vitamin E and with metyrapone tended to dissociate ethylene production from lipid peroxidation. These experiments illustrate both the experimental potential and the experimental pitfalls inherent in the use of KMB or methional to detect radical production *in vivo*.

MATERIALS AND METHODS

The sodium salt of alpha-keto-gamma-methiolbutyric acid (KMB) and metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone) were purchased from the Sigma Chemical Co. (St. Louis, MO). CCl_4 and NaCl were reagent grade. Heavy mineral oil, used as the vehicle for CCl_4 administration, was from Purepak Pharmaceuticals (Elizabeth, NJ). Ethane and ethylene calibration standards were purchased from Alltech Associates, Deerfield, IL. Injectable preparations of vitamin E (*dl*-alpha-tocopherol, 50 mg/ml) and placebo for vitamin E were the gifts of Hoffmann–LaRoche Inc. (Nutley, NJ). Each milliliter of placebo or vehicle for vitamin E contained 0.1 ml ethanol, 0.1 ml propylene glycol, 0.1 ml Emul-

* Address all correspondence to: Dr. Glen D. Lawrence, Institute of Human Nutrition, Columbia University College of Physicians & Surgeons, 630 West 168 St., New York, NY 10032.

phor EL-620, 0.01 ml benzyl alcohol, 0.3 mg sodium acetate trihydrate, 2.5 mg acetic acid, 9 mg NaCl and 0.1 mg Na_2EDTA adjusted to pH 4 with dilute NaOH. Air zero grade (hydrocarbon-free air; i.e. less than 0.5 ppm) was from Linde (South Plainfield, NJ).

Male Swiss–Webster mice (Perfection Breeders, Douglasville, PA) weighing 25–35 g were maintained on Purina lab chow with free access to water. Food was withheld overnight prior to hydrocarbon exhalation studies. Animals received one of the following four treatments by i.p. injection prior to hydrocarbon exhalation analysis. One group received 3.0 g CCl_4/kg body wt (300 mg CCl_4/ml mineral oil), followed 20 min later by 0.6 g KMB/kg body wt (60 mg KMB/ml in water). A second group received 3.0 g CCl_4/kg body wt, followed 20 min later by 1.0 ml isotonic saline/100 g body wt. A third group received 1.0 ml mineral oil (vehicle for CCl_4 injections)/100 g body wt followed 20 min later by 0.6 g KMB/kg body wt. A fourth group received 1.0 ml mineral oil/100 g body wt followed 20 min later by 1.0 ml saline/100 g body wt. In other experiments, a higher dose of 1.0 g KMB/kg body wt was used. All KMB solutions were freshly prepared just prior to injection.

In some experiments, animals received three daily injections of vitamin E (100 mg/kg body wt) or placebo for vitamin E (equivalent volume of vehicle), with the last injection administered 30 min prior to CCl_4 or mineral oil injection. In other experiments, animals received 150 mg metyrapone/kg body wt in 20% aqueous ethanol solution (ethanol dose, 1.6 g/kg) 1 hr prior to CCl_4 or KMB administration.

Animals were placed in the breath collection chambers (described in Ref. 14) immediately after the last injection. The chambers were purged with hydrocarbon-free air for 2 min; they were then sealed, and the air circulating pump was turned on for 3 min prior to removing the first 30-ml sample for hydrocarbon analysis. This first sample was used as the zero time value for the chamber, and all subsequent samples were measured as changes in hydrocarbon levels with respect to zero time. The total volume of the breath collection system was 0.75 l. A 30-ml sample was removed at 20-min intervals for hydrocarbon analysis. Ethane and ethylene were quantitated by gas chromatography on a Hewlett–Packard model 5750 gas chromatograph equipped with a Chemical Data Systems model 310 concentrator, a 1.8 m stainless steel column packed with Porapak N (60/80 mesh), and a flame ionization detector. The concentrator traps were packed with activated charcoal for adsorbing ethane and ethylene at room temperature, and they were programmed for desorption at 250°. The helium carrier gas flow rate was 20 ml/min, and column oven temperature was 70°. The entire 30-ml sample was injected into the gas concentrator and flash desorbed onto the column. Ethane and ethylene were quantitated by comparison with standards. (See Ref. 14 for details of the breath collection procedure).

In vitro studies were performed with fresh mouse liver slices prepared from vitamin E-pretreated and placebo-pretreated mice. Vitamin E and placebo were administered as described above. Mice were killed by cervical dislocation, and th livers were excised and placed in ice-cold isotonic saline. Pieces of liver (0.51 ± 0.02 g) were diced into 2–3 mm cubes with a stainless steel razor blade and placed into 4.9 ml of ice-cold isotonic saline containing 2 mg glucose/ml. Incubations were done in 25-ml Erlenmeyer flasks sealed with rubber septa, which allowed for sampling by syringe for headspace analysis of hydrocarbon gases. Separate flasks received additions of 20 μl CCl_4 or 100 μl of 0.5 M KMB solution (final KMB concentration was 10 mM) or both, and were placed on a shaking water bath at 37°. Headspace gas samples (0.5 ml) were removed at 20-min intervals and analyzed by direct injection into the gas chromatograph (bypassing the concentrator) under the conditions described above.

RESULTS

Measurement of ethane production was used to monitor *in vivo* lipid peroxidation. Figure 1 shows that CCl_4 administration stimulated the production of ethane compared to mice receiving the mineral oil vehicle alone. These results are in agreement with earlier studies [15–17]. Animals receiving KMB (0.6 g/kg) after CCl_4 produced slightly less ethane than those receiving saline after CCl_4, but the difference was not statistically significant. Administration of KMB did not cause any significant change in ethane production by control mice injected with the mineral oil vehicle.

Figure 2 shows that KMB administration to mice resulted in the production of ethylene. When mice were pretreated with CCl_4, ethylene production was increased. A greater than 6-fold increase in ethylene production was seen at 60 min compared to animals receiving saline after CCl_4. Ethylene production following KMB was 2-fold greater in CCl_4-treated mice compared to mice receiving the mineral oil vehicle. These results demonstrate a background production of ethylene from KMB, with clear stimulation of ethylene production by injection of CCl_4.

To determine whether the background ethylene production from KMB was due to spontaneous, low-

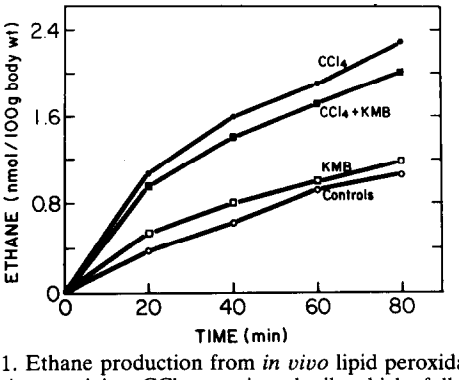

Fig. 1. Ethane production from *in vivo* lipid peroxidation by mice receiving CCl_4 or mineral oil vehicle followed 20 min later by KMB or saline. Zero time is the point of KMB or saline injection. Key: (●) CCl_4 followed by saline (N = 6); (■), CCl_4 followed by KMB (N = 6); (○) mineral oil followed by saline (controls, N = 5); and (□) mineral oil followed by KMB (N = 6). CCl_4 dose, 3.0 g/kg body wt (300 mg CCl_4/ml in mineral oil vehicle). KMB dose, 0.6 g/kg body wt (60 mg KMB/ml in water).

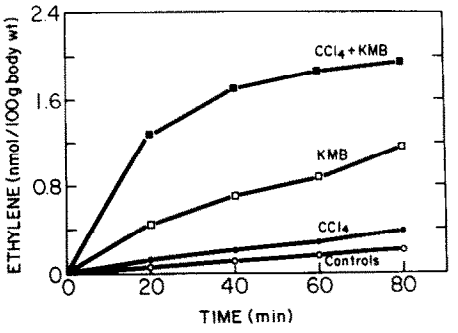

Fig. 2. Ethylene production from KMB by the mice in Fig. 1. Ethane and ethylene were monitored simultaneously for each animal. The same symbols apply as for Fig. 1.

level, lipid peroxidation (with accompanying generation of radical intermediates), animals were injected with large doses of vitamin E (100 mg/kg body wt) daily for 3 days prior to KMB administration. These animals received the same treatment regimen for CCl_4 and KMB as before, except that the dose of KMB was raised to 1.0 g/kg instead of 0.6 g/kg body wt; the higher dose of KMB amplified the production of ethylene. Table 1 shows the production of ethane and ethylene in 60 min by non-pretreated, placebo-pretreated and vitamin E-pretreated mice that subsequently received one of the four treatment regimens for CCl_4 and KMB (columns 2, 3 and 4). Values obtained with non-pretreated mice receiving the lower dose of 0.6 g KMB/kg are included for comparison (first column). The higher dose of KMB increased the production of ethylene (lines 6 and 8, compare the first and second columns), but had no significant effect on ethane (lines 2 and 4). Increased production of ethylene was seen with CCl_4 and KMB, compared to KMB alone, in each pretreatment protocol (compare lines 6 vs 8 in columns 1–4); the differences were statistically significant (superscripts ‡, § and ‖), except for the placebo-pretreated group.

As shown in earlier studies [15, 16], vitamin E, an agent that suppresses CCl_4-induced lipid peroxidation, also suppressed the consequent exhalation of ethane (line 3, superscript †; vitamin-E-pretreated, 1.01 ± 0.06 vs placebo-pretreated, 2.41 ± 0.22 nmoles/100 g in 60 min). Furthermore, as shown earlier in Fig. 1 for non-pretreated mice, KMB administration did not have any significant effect on ethane production (compare lines 1 vs 2, and lines 3 vs 4, in the second, third, and fourth columns).

Table 1. Ethane and ethylene production by mice receiving CCl_4 or mineral oil followed by KMB or saline*

	Hydrocarbon production (nmoles/100 g in 60 min)				
	KMB = 0.6 g/kg (where indicated)	KMB = 1.0 g/kg (where indicated)			
	Untreated	Untreated	Placebo pretreated	Vitamin E pretreated	Metyrapone treated
		Ethane production			
1. Saline alone		0.94 ± 0.12 (5)	0.60 ± 0.10 (5)	0.50 ± 0.06 (5)	
2. KMB alone	0.97 ± 0.17 (6)	0.76 ± 0.10 (6)	0.73 ± 0.08 (8)	0.58 ± 0.03 (8)	0.87 ± 0.36 (3)
3. CCl_4 + saline		1.90 ± 0.16¶ (6)	2.41 ± 0.22† (5)	1.01 ± 0.06† (5)	1.03 ± 0.34¶ (2)
4. CCl_4 + KMB	1.71 ± 0.15 (6)	1.79 ± 0.11 (6)	2.78 ± 0.24 (6)	0.94 ± 0.12 (6)	1.17 ± 0.12 (4)
		Ethylene production			
5. Saline alone		0.17 ± 0.02 (5)	0.34 ± 0.02 (5)	0.39 ± 0.08 (5)	
6. KMB alone	0.87 ± 0.16‡ (6)	1.47 ± 0.35‖ (6)	1.94 ± 0.50 (8)	1.60 ± 0.26§ (8)	1.79 ± 0.28** (3)
7. CCl_4 + saline		0.29 ± 0.08 (6)	0.36 ± 0.06 (5)	0.29 ± 0.08 (5)	0.12 ± 0.04 (2)
8. CCl_4 + KMB	1.86 ± 0.17‡ (6)	2.37 ± 0.10‖ (6)	2.53 ± 0.22 (6)	3.09 ± 0.43§ (6)	2.96 ± 0.35** (4)

* Ethane and ethylene were monitored simultaneously by gas chromatography for each mouse. Number of animals (N) is shown in parentheses below the mean ± S.E.M. for each group. The first column presents data from experiments with 0.6 g KMB/kg; the remaining columns show data for experiments with 1.0 g KMB/kg. Animals received: "Saline alone"—1.0 ml saline/100 g body wt; "KMB alone"—1.0 g KMB/kg body wt; "CCl_4 + saline"—3.0 g CCl_4/kg body wt followed by 1.0 ml saline/100 g body wt 20 min later; "CCl_4 + KMB" = CCl_4 followed by KMB 20 min later. Untreated mice received no pretreatment before CCl_4 and/or KMB. Placebo-pretreated and vitamin E-pretreated mice received vehicle alone or with vitamin E (100 mg/kg) daily for 3 days, prior to CCl_4 and/or KMB. Metyrapone-treated mice received 150 mg metyrapone/kg body wt 1 hr prior to CCl_4 and/or KMB. See Materials and Methods for further details.

†–** Some of the more pertinent comparisons are indicated by symbol superscripts, as follows: † = $P < 0.001$, ‡ = $P < 0.005$, § = $P < 0.01$, ‖ and ¶ = $P < 0.05$, and ** = P between 0.05 and 0.10, where comparisons are made between values bearing the same symbols.

The lower part of Table 1 shows that vitamin E administration had virtually no effect on ethylene production compared to placebo-injected groups (columns 3 vs 4) for either saline-injected mice or KMB-injected mice receiving prior injections of CCl_4 or mineral oil vehicle (i.e. in all four groups; lines 5–8). Non-pretreated mice (no vitamin E or placebo pretreatment, column 2) were given CCl_4 or mineral oil, followed by KMB (1 g/kg) or saline, to see if administration of the placebo for vitamin E (column 3) had any effect on ethylene production. There were no significant differences in KMB-stimulated ethylene production in non-pretreated, placebo-pretreated, or vitamin E-pretreated mice (line 6, columns 2, 3 and 4; 1.47 ± 0.35, 1.94 ± 0.50 and 1.60 ± 0.26 nmoles/100 g, respectively); similarly, there were no significant differences when mice received both CCl_4 and KMB (line 8).

Administration of KMB after CCl_4 did not have a significant effect on ethane production (line 4 vs line 3, columns 2, 3 and 4), suggesting that KMB did not affect CCl_4-stimulated lipid peroxidation. However, placebo-pretreated mice exhaled more ethane than non-pretreated mice (column 3 vs column 2, lines 3 and 4). One possible explanation for the elevated ethane production by CCl_4-treated mice receiving placebo, compared to non-pretreated mice, is that the alcohols (ethanol, propylene glycol, Emulphor EL-620 and benzyl alcohol) in the vehicle may have induced the microsomal mixed function oxidase system, giving rise to increased CCl_4 stimulation of lipid peroxidation, similar to that observed with phenobarbital induction of the microsomal proteins [15, 17].

In vitro studies were performed with mouse liver slices in order to determine whether the whole body *in vivo* measurements could be related to liver. Mice were pretreated with vitamin E or placebo, as for the *in vivo* studies, and then liver slices were prepared. CCl_4-stimulated ethane production and KMB-dependent ethylene production were studied *in vitro*. The results shown in Table 2 reveal phenomena similar to those observed *in vivo*. Vitamin E pretreatment strongly suppressed CCl_4-stimulated ethane production, but had no effect on ethylene production from KMB. Addition of KMB to actively peroxidizing liver slices that were preincubated with CCl_4 for 40 min resulted in a burst of ethylene production from both vitamin E-pretreated and placebo-pretreated liver slices. The magnitude of the CCl_4-stimulated increase in ethylene production from KMB was equal in both vitamin E- and placebo-pretreated preparations, even though vitamin E pretreatment continued to suppress ethane production. The results obtained with vitamin E *in vivo* and with liver slices *in vitro* tend to dissociate hepatic lipid peroxidation from KMB-dependent ethylene formation.

Some mice were treated with metyrapone (150 mg/kg body wt), an inhibitor of cytochrome P-450, 1 hr prior to CCl_4 administration. Results are shown in Table 1 (last column). Metyrapone inhibited the CCl_4-induced ethane production (column 5 vs column 2, line 3, superscript ¶) to about the same extent as vitamin E (column 4 vs column 3, line 3). Similar results were seen when CCl_4 and KMB were injected (line 4). Metyrapone treatment caused about 90% inhibition of the CCl_4-stimulated ethane production. On the other hand, metyrapone did not inhibit the CCl_4-stimulated production of ethylene from KMB (line 8, column 5 vs column 2). Metyrapone administration caused some increase in ethylene production from KMB by itself (line 6, column 5 vs column 2), and from CCl_4 plus KMB (line 8), although these increases were not significant. This increase in ethylene exhalation as a result of metyrapone treatment may be due to inhibition of ethylene metabolism by the mixed function oxidase system, since it has been shown that ethylene is metabolized both *in vivo* [14] and *in vitro* [18]. Ethylene production from KMB was stimulated by CCl_4 to a similar extent in the metyrapone-pretreated mice (line 8 vs 6, column 5, superscript **) and in the non-pretreated groups (line 8 vs 6, column 2). These

Table 2. Ethane and ethylene production from mouse liver slices*

Additions	Pretreatment	Ethane (nmoles/flask/40 min)	Ethylene (nmoles/flask/40 min)
None (controls)	Placebo	0.11	ND
	Vitamin E	0.05	ND
CCl_4	Placebo	0.70 ± 0.10	ND
	Vitamin E	0.27 ± 0.03	ND
KMB	Placebo	0.15 ± 0.06	0.65 ± 0.06
	Vitamin E	0.06 ± 0.01	0.67 ± 0.03
CCl_4 at 0 min	Placebo	0.98 ± 0.08	3.51 ± 0.10
+ KMB at 40 min†	Vitamin E	0.16 ± 0.03	3.52 ± 0.26

* Ethane and ethylene production were measured in the headspace gas of liver slices incubated in sealed 25-ml Erlenmeyer flasks, as described in Materials and Methods. Ethane and ethylene were measured simultaneously, and each value in the table is the mean ± S.E.M. of three replicates, except for controls which are the average of duplicates. ND = not detectable.

† Addition of KMB at 40 min was made by syringe directly through the rubber seal of the Ehrlenmeyer flasks. For these samples, the 40-min readings were subtracted from those at 80 min to provide the rate of hydrocarbon gas formation over 40 min.

results confirm the dissociation between hepatic lipid peroxidation and KMB-dependent ethylene formation.

In other experiments, administration of indomethacin (2 mg/kg) to block potential radical formation by the cyclooxygenase pathway was without effect on the background production of ethylene from KMB (data not shown).

We also tested methional, used in place of KMB, in experiments with CCl_4. Methional is a less polar and more lipophilic scavenger of radicals. Methional is not water soluble in high concentration, and was administered as a suspension in mineral oil. Methional was much more toxic to mice than KMB. Therefore, a lower dose of 0.3 g/kg was used. This dose of methional by itself resulted in an ethylene response (0.80 ± 0.45 nmoles/100 g in 60 min, N = 3) comparable to that observed with 0.6 g KMB/kg body wt (0.87 ± 0.40, column 1, line 6). CCl_4 administration 20 min prior to methional gave only a marginal stimulation of ethylene production (0.92 ± 0.35 nmole/100 g, N = 4). Although the dose of methional (0.3 g/kg, or 2.9 mmoles/kg) was somewhat less than that for KMB (0.6 g/kg, or 3.5 mmoles/kg), these results suggest that the more lipophilic scavenger was no more effective than KMB at intercepting radicals generated during lipid peroxidation. These two thioethers have comparable activities in flavin-photosensitized reactions [10] and, presumably, other radical mediated reactions.

DISCUSSION

This report of *in vivo* production of ethylene from KMB is the first demonstration that we know of this phenomenon in mammals. The *in vitro* metabolism of KMB to products other than ethylene by rat liver homogenates and by partially purified enzymes has been described [19, 20]. Fu *et al.* [21] showed that rat liver extracts were capable of forming ethylene from methionine. Yang *et al.* [10] demonstrated nonenzymatic photochemical production of ethylene, carbon dioxide, formic acid, methyldisulfide and ammonia from methionine in the presence of flavin mononucleotide. We have observed that addition of CCl_4 to solutions of KMB under ambient fluorescent lighting in the laboratory stimulates a small amount of ethylene production; stimulation by CCl_4 does not occur in the dark (unpublished observation).

The results presented here demonstrate that *in vivo* ethylene production is increased in mice by the administration of KMB. There is further stimulation in ethylene production by CCl_4 administration. However, the stimulation is not sensitive to inhibitors of CCl_4-induced lipid peroxidation, viz. vitamin E and metyrapone. Dumelin and Tappel [22] have shown that the yield of volatile hydrocarbons (ethane and pentane) from peroxidizing polyunsaturated fatty acids is very small (on the order of 1% in mole equivalents) compared to other measurements of lipid peroxidation (e.g. diene conjugation, fatty acid decrease or peroxide value). The yield of ethylene from KMB in CCl_4-injected mice was similarly small when compared to the yield of ethane (Table 1). We had expected a greater yield of ethylene because KMB should intercept many more intermediate radicals during lipid peroxidation than merely those that give rise to ethane and pentane. A greater yield of ethylene, relative to ethane, was observed in experiments conducted with CCl_4 and KMB *in vitro* (liver slices, Table 2), but not *in vivo*.

Several factors should be considered in assessing ethylene production from KMB *in vivo*. (1) Competitive reactions of intermediate radicals with tissue constituents may limit the yield. (2) Ethylene can be metabolized by the mixed function oxidase system of liver [14, 18], with resultant lowering of observed yields. On the other hand, agents that compete with or inhibit the mixed function oxidase can elevate the yields. (3) KMB may be rapidly metabolized in liver, or may not penetrate well into liver. Moreover, the polar KMB molecule may be excluded from hydrophobic regions of the membrane or organelle where lipid peroxidation is taking place. However, it is noted that the less polar, lipophilic analog, methional, did not produce greater yields of ethylene. (4) Stimulation of ethylene production by CCl_4 may represent an extrahepatic phenomenon. For example, peritoneal macrophages or blood-borne leukocytes may be responsible for the background production of ethylene from KMB, and CCl_4 may augment radical production by these cell types. However, one must bear in mind that CCl_4 also stimulated ethylene production from KMB in isolated mouse liver slices *in vitro* (Table 2); Feierman and Cederbaum (personal communication) have observed a similar stimulation with isolated hepatocytes. (5) Ascorbic acid, which is omnipresent in living organisms, will stimulate the production of ethylene from KMB in the presence of trace amounts of metal ions, such as iron or copper. This may account for some of the background ethylene produced from KMB in untreated animals. (6) The possibility that CCl_4 may elicit a nonspecific solvation effect for transport of KMB into cells cannot be ruled out. However, an effect on outward transport of hydrocarbon gases is ruled out because the enhanced ethane production is blocked by tocopherol or metyrapone.

It is clear that both vitamin E and metyrapone suppress the CCl_4-stimulated production of ethane (Table 1). These results indicate a suppression of lipid peroxidation by both of these agents: vitamin E by intercepting the radical chain intermediates of lipid peroxidation, and metyrapone by inhibiting the cytochrome P-450-mediated initiation of lipid peroxidation by CCl_4. Wendel and Feuerstein [23] have shown that metyrapone suppresses paracetamol (acetaminophen)-stimulated ethane exhalation (lipid peroxidation) by mice *in vivo*. In our experiments, metyrapone had no effect on either ethylene production from KMB administration by itself, or from CCl_4 plus KMB.

If KMB were to intercept alkoxyl radicals or other free radicals in the lipid peroxidation chain reaction, one would expect that ethane production would, of necessity, also be decreased significantly. But, this was not observed (Fig. 1 and Table 1). These results, and the failure of vitamin E or metyrapone to suppress ethylene production from KMB (either with or without CCl_4), indicate that KMB-derived ethylene

does not monitor free radical intermediates during hepatic lipid peroxidation *in vivo*.

This study was initiated in an attempt to detect *in vivo* production of free radicals. The initiation of lipid peroxidation by CCl_4 was chosen as an extensively studied model for radical production *in vivo*. KMB and methional were the radical scavengers of choice because they produce a volatile hydrocarbon (ethylene) that is readily measured in the expired air from an experimental animal. The method is also non-invasive. This study does illustrate KMB-dependent (and methional-dependent) formation of ethylene from endogenous sources, with stimulation of ethylene production by CCl_4. However, the results with vitamin E and metyrapone dissociate ethylene production from lipid peroxidation. Both agents suppressed ethane production (an *in vivo* index of lipid peroxidation); however, neither agent suppressed ethylene production. Although the results of this study are not definitive, they do represent a state-of-the-art approach to the study of radical-mediated processes *in vivo*. Further studies are warranted to elucidate the endogenous sources of KMB-dependent ethylene production, and the nature and meaning of the increments seen with CCl_4 both *in vivo* and *in vitro*.

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