Concentrating Breath Samples Using Liquid Nitrogen: A Reliable Method for the Simultaneous Determination of Ethane and Pentane

Mitchell D. Knutson and Fernando E. Viteri[1]

Department of Nutritional Sciences, 17 Morgan Hall, University of California, Berkeley, California 94720

The measurement of ethane and pentane in breath offers a sensitive and noninvasive means to assess *in vivo* lipid peroxidation in animals and humans. However, numerous technical obstacles inherent in collecting and concentrating air-breath samples have limited the wider application of these measurements for the assessment of *in vivo* lipid peroxidation. We have developed a relatively simple, inexpensive, rapid, and reliable method to collect, concentrate, and measure breath ethane and total-body pentane from rats. This method, which concentrates alkanes from 4 liters of collected air-breath on adsorbant cooled to −174°C, was found to be superior to similar cryofocusing techniques at −130°C, which fail to effectively trap highly volatile ethane from large volumes of air. We found ethane evolves predominantly through breath, whereas a significant amount of pentane evolves from sources other than breath. Mean evolution rate for ethane was 1.08 pmol/100 g body wt/min. Pentane evolution rates displayed more inter-rat and day-to-day variability with a mean of 0.52 pmol/100 g body wt/min. We also found that excreted rat feces exude large amounts of ethane and pentane. © 1996 Academic Press, Inc.

Lipid peroxidation, the free radical-catalyzed oxidation of polyunsaturated fatty acids, is associated with numerous pathological conditions, including atherosclerosis and cancer (1). Ethane and pentane are the main volatile hydrocarbons formed during the breakdown of peroxidized lipids (2), and because they are exhaled through breath, their measurement offers a noninvasive means to assess whole-body lipid peroxidation in animals and humans (3). Although ethane and pentane are considered to be sensitive indicators of *in vivo* lipid peroxidation, their evolution[2] in the process of lipid peroxidation represents only a minor pathway (2), and their concentrations in breath are usually considerably lower than the detection limit of most gas chromatographic methods. The breath alkanes must therefore be concentrated.

Cryofocusing techniques are commonly used to concentrate breath alkanes from small animals (4–8). First, a sample of air is collected after it has been passed through an open-flow respiratory chamber containing the animal (5) or through a section of a chamber that has the animal's head hermetically isolated from the rest of the body (4). The air-breath sample is then scrubbed of water vapor and carbon dioxide and passed through a cold adsorbing material, which traps alkanes but not nitrogen and oxygen. Last, the alkanes are desorbed from the adsorbant with heat into a small volume of nitrogen.

Closed, air-tight rebreathing systems have also been used to concentrate breath samples from small animals (9–12). In these systems, an animal is placed in a known volume of air for several hours while removing carbon dioxide and water vapor and supplying fresh oxygen. However, because evolved pentane can be significantly metabolized by the animal during the collection process (14, 15), closed rebreathing systems are not suitable to measure the true evolution rate of pentane.

The differences in physical properties between ethane and pentane also complicate their simultaneous collection, concentration, and analysis (3). For example, pentane may condense on system components during collection because its boiling point (+36°C) is higher

---

[1] To whom correspondence should be addressed. Fax: (510) 642-0535.

[2] "Evolution" of alkanes is defined as the process by which alkanes are formed and released from the body through breath and/or other routes.
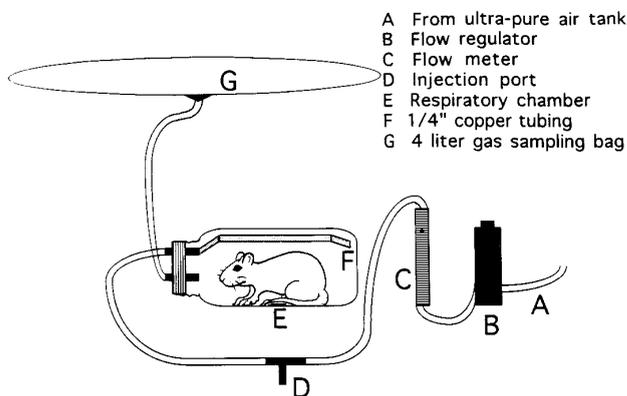
A From ultra-pure air tank
B Flow regulator
C Flow meter
D Injection port
E Respiratory chamber
F 1/4" copper tubing
G 4 liter gas sampling bag

FIG. 1. Breath collection system.

than usual ambient temperatures. Alternatively, ethane, because of its low boiling point ($-89°C$) is difficult to cryofocus, even when adsorbants are at low temperatures (ca. $-130°C$). As for chromatographic analysis, the differences in molecular weights of ethane and pentane often results in undesirably large differences in retention times.

In response to the need to simultaneously collect and effectively concentrate ethane and pentane from small animals, we have developed a method that collects air-breath samples with an open-flow respiratory chamber, concentrates the alkanes with liquid nitrogen, and permits the GC analysis of both ethane and pentane in under 10 min.

MATERIALS AND METHODS

Breath Collection System Materials

The breath collection system is shown in Fig. 1. An adjustable stainless steel diaphragm flow regulator was used to ensure a constant flow rate. The open-flow respiratory chamber was made from a 1-liter Mason jar purchased from the local hardware store. The connections through the lid of the respiratory chamber were made from $\frac{1}{4}''$ Swagelok bulkhead male connectors. The copper tubing ($\frac{1}{4}''$ OD $\times$ 0.190" ID), nuts ($\frac{1}{4}''$), and Swagelok ferrules ($\frac{1}{4}''$) were purchased from Alltech Associates, Inc. (Deerfield, IL). Connection tubing was made from Tygon ($\frac{1}{4}''$ OD $\times \frac{1}{8}''$ ID) obtained from Fisher Scientific (Santa Clara, CA). All tubing connections were secured with Parafilm M laboratory film from Fisher Scientific (Santa Clara, CA) to ensure the firmness of the hermetic collection. The calibrated flowmeter (air range, 1–280 ml/min) and Tedlar gas sampling bags ($12'' \times 12'' \times 2''$) with on/off valve were purchased from Baxter Diagnostics Inc. (McGaw Park, IL).

Cryofocusing Materials

Activated alumina (80/100 mesh) and Porasil C (80/100 mesh), from Alltech Associates, Inc., were used as the alkane adsorbants in the U-loop (Fig. 2). The 120 cc, $\frac{1}{8}''$ in-line gas purifier, copper tubing ($\frac{1}{4}''$ OD $\times$ 0.190" ID), stainless steel tubing ($\frac{1}{8}''$ OD $\times$ 0.085" ID), and 3-way ball valves (1"4) were also obtained from Alltech Associates, Inc. Gas-tight glass syringes (SGE liquid/gas, 50 and 100 ml) were purchased from Supelco (Bellafonte, PA). Indicating Drierite, soda lime (4–8 mesh), Tygon tubing ($\frac{1}{4}''$ OD $\times \frac{1}{8}''$ ID), and Dewar flask (350 ml) were obtained from Fisher Scientific. Liquid nitrogen and a Gillette Supermax Styler 1200 blowdryer were obtained locally. Vacuum pump with gauge and regulator (maximum vacuum, 23.2" Hg) was purchased from Cole Parmer (Niles, IL). Temperatures were determined with a Barnant 115 thermocouple thermometer which was obtained from Fisher Scientific.

Gases

Ultra-pure air (Air, Ultra-Zero) and the alkane standard mixture (0.5 ppm ethane/0.5 ppm pentane) were obtained from Matheson (Newark, CA). Nitrogen, oxygen, and hydrogen were obtained from the University of California Storehouse (Berkeley, CA).

Gas Chromatography

A Varian 3700 gas chromatograph (Varian Instruments, Palo Alto, CA) fitted with a flame ionization detector and a 15 m $\times$ 3.18 mm OD stainless steel column packed with 80–100 mesh Porasil C (Alltech) was used for the analysis of ethane and pentane. Carrier gas temperature was set isothermally at 40°C with a flow rate of 60 ml/min. Oxygen and hydrogen flow rates to the detector were 50 ml/min and 40 ml/min, respectively. Temperature of the injection port was 70°C, and the detector oven was set at 200°C. The detector output was connected to a chart recorder, model L-101 (Quintron Instrument Co., Inc., Milwaukee, WI). The gas chromatograph was calibrated daily with 4 ml alkane standard diluted to 50 ml with nitrogen.

Standard Curves

Standard curves (Fig. 3) were prepared for both ethane and pentane by adding various amounts of alkane standard (0, 41, 82, 164, and 328 pmol) to 4 liters of ultra-pure air and concentrating the sample as described under *Concentration of Alkanes from Air-Breath Samples.*

Recovery of Alkanes from Breath Collection System

The breath collection system was first flushed with ultra-pure air (ca. 600 ml/min) for 25 min. Then at $t = 0$, after the flow rate was adjusted to 200 ml/min, 90 pmol of alkane standard were added to the system through the injection port (Fig. 1) and a 4-liter volume
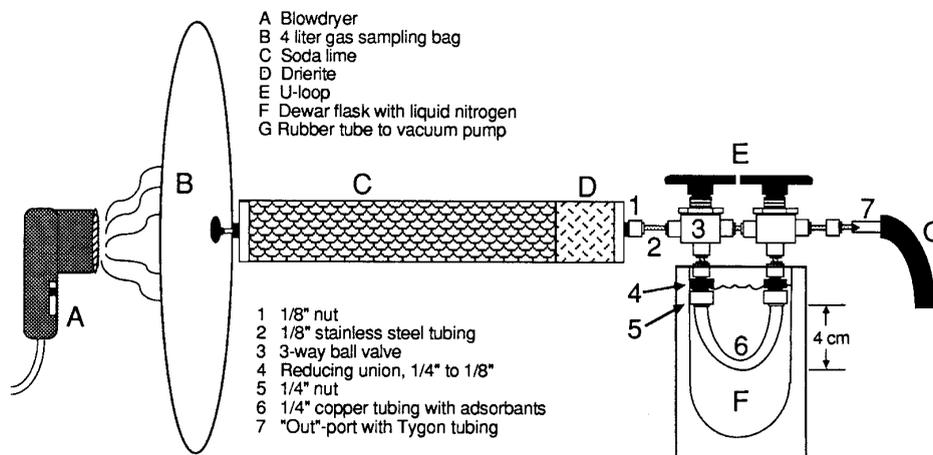
FIG. 2. Set-up to concentrate alkanes at $-174°C$. The $\frac{1}{4}''$ copper tubing of the U-loop is tightly packed (from left to right on the above diagram) with 0.6 g Porasil-C and 0.3 g activated alumina.

was collected in 20 min. Gases were collected in the Tedlar bags and then concentrated and analyzed as described below. For these studies an electric heating pad (set on low setting) was placed around the open-flow respiratory chamber to simulate the heat produced from a rat in the chamber. In addition, an aluminum foil mock rat was placed in the animal chamber to compensate for the volume of a 250-g rat.

Animals

Male Sprague–Dawley rats, weighing between 200 and 300 g were obtained from Bantin Kingman (Fre-

FIG. 3. Standard curves obtained from concentrating various amounts of alkane standard added to 4 liters of ultra-pure air. All points in duplicate. Values on the x-axis represent the amount of alkane standard analyzed in 5 ml of a total of 50 ml that was concentrated from the 4 liters.

mont, CA). Rats were housed singly in hanging cages and given free access to water and Purina Rat Chow No. 5012. Rats were fasted for 12 h prior to collecting air-breath samples.

Collection of Air-Breath Samples

Because rat feces exude significant amounts of ethane and pentane, the rat must not defecate in the respiratory chamber during the collection procedure. We have found that agitating the rat by gently shaking its tail quickly promotes defecation prior to placing the rat in the chamber. The rat is then placed in the respiratory chamber (Fig. 1) and ultra-pure air is passed through the system (ca. 600 ml/min) for 25 min in order to flush the breath collection system and the rat's lungs of ambient hydrocarbons and to acclimate the rat to the confined chamber and the ultra-pure air. A 4-liter air-breath sample is collected at a flow rate of 200 ml/min into a gas sampling bag and stored at room temperature until analysis. The glass respiratory chamber is washed with warm soapy water, rinsed well, and dried before collecting from another rat. Gas sampling bags are routinely flushed five times with nitrogen before use.

Concentration of Alkanes from Air-Breath Samples

Fresh Drierite (ca. 25 g) and soda lime (ca. 65 g) are added to the in-line gas purifier and a strong stream of nitrogen is passed through for 20 min to remove contaminant alkanes. Before concentrating air-breath samples, we routinely concentrate a 4-liter sample of ultra-pure air to ensure that the Drierite/soda lime unit is adequately flushed of contaminant alkanes and that all connections are air-tight. The Drierite and soda lime need to be replaced after concentrating four air-breath
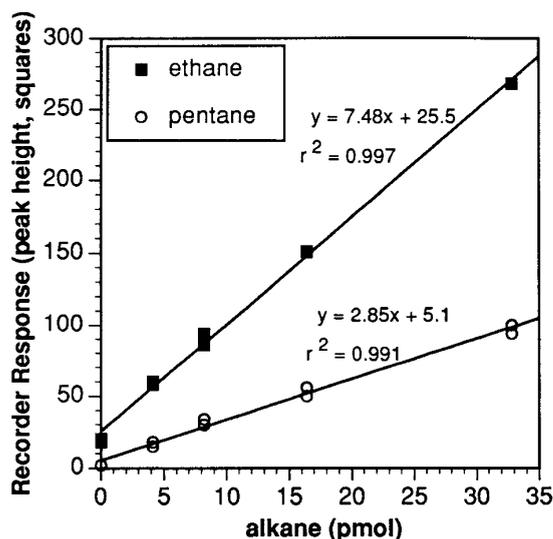
samples. If the soda lime is not changed, carbon dioxide that is injected into the GC will interfere with the ethane peak.

The set-up for the concentration of air-breath samples is shown in Fig. 3, and the following trap and purge procedure is used. The closed U-loop is first cooled for exactly 2 min by placing it in a Dewar flask filled to the top with liquid nitrogen. Liquid nitrogen is occasionally added to the Dewar flask to ensure that the adsorbant materials in the U-loop are completely submerged during the adsorption of alkanes. The gas sampling bag is connected to the Drierite/soda lime unit by pushing the small piece of Tygon tubing snugly onto the $\frac{1}{8}''$ ferrule that is at the end of the left arm of the Drierite/soda lime unit (Fig. 2). Connections such as these are routinely secured with a strip of Parafilm to ensure they are completely air-tight. Next, the vacuum pump is turned on, the valves of the U-loop are opened, and the contents of the bag are pulled through the loop. About 3 min are needed to pass 4-liter samples through the cold U-loop. During the entire process of emptying the gas sampling bag it is slightly warmed with the blow-dryer on the 300-watt setting in order to evaporate any pentane that may have condensed on the inner walls of the bag during storage of the samples.

Immediately after the bag is voided, the valves of the U-loop are closed, the vacuum pump connection is replaced by a 50-ml glass syringe, and the Drierite/soda lime container is disconnected. The right-side valve is then opened and the U-loop is taken out of the liquid nitrogen and is placed in a hot-oil bath at $+170°C$ for exactly 3 min. Upon heating, usually less than 30 ml of condensed gas expands into the syringe. Nitrogen is passed through the U-loop to purge any hydrocarbons that are still in the loop into the syringe, up to a total volume of 50 ml. At least 10 ml are required to flush all of the ethane and pentane out of the loop. Of the total 50 ml purge volume, 5 ml is injected into the GC for quantitation. Amounts of ethane and pentane (in pmol) are derived from the standard curves in Fig. 3.

Statistical Analyses

All statistical analyses were performed using StatView 4.1 software (Abacus Concepts, Inc., Berkeley, CA).

RESULTS AND DISCUSSION

Validation of the Collection and Concentration of Alkanes

Table 1 shows that 4 liters of ultra-pure air contains trace amounts of ethane and pentane. Other investigators have attempted to eliminate alkanes from ultra-pure air sources by scrubbing the effluent stream with adsorbants such as activated charcoal (5) or mo-

lecular sieve 5A (4, 9). We decided against this measure because of the risk of ethane contamination by these adsorbants if they are not scrupulously flushed or if they become saturated with alkanes. Moreover, we found background alkane levels to be very consistent in ultra-pure air (even from different tanks from the same supplier) and that the amounts of ethane and pentane in ultra-pure air (Fig. 4A) are considerably lower than those from air-breath samples from normal rats (Fig. 4B).

No differences in background alkane levels were found if 4 liters of ultra-pure air was collected either directly into the gas sampling bag or through the breath collection system (Table 1). This demonstrates that the system is hermetic and that there is no alkane contamination from the materials used in the breath collection system. Likewise, over 90% of 90 pmol of standard alkane mixture was recovered, indicating that there was essentially no alkane loss from the system. The 7% of pentane not recovered 20 min after it was injected into the system suggests that some pentane condensed on system components.

In any system used to collect ethane and pentane, numerous precautions should be followed in order to avoid the many sources of ethane and pentane contamination. We have found Drierite and soda lime to contribute ethane and pentane (which, however, could be removed by flushing the in-line gas purifier for 20 min with a strong nitrogen stream). Several nitrogen tanks were also found to be contaminated with pentane. Contamination with ambient air is also a problem if the Tedlar bags have even the slightest leak. Although the bags can be reused many times to collect air-breath samples, we have found that some bags develop leaks over time. We therefore recommend the periodic testing of the bags and the collection of samples at least in two different bags.

Figure 3 shows that a linear response for concentrating various amounts of ethane and pentane is obtained for a large range of alkane concentrations. For routine analyses of normal rats (ca. 200 g), standard curves that span the expected range (0–8.2 pmol) are prepared. The variation is negligible for repeated standard curves (data not shown).

Alkane Evolution by the Rat

Table 2 shows the inter-rat variation and day-to-day reproducibility in measuring rat breath alkanes. These values are in the range reported by other authors (4, 15, 16). No significant differences in ethane excretion rates were measured between four different rats that were studied on the same day or when the same rat was studied on different days. In contrast, pentane was found to have significant variability between rats and between days for the same rat. The variability is most

Alkane Levels Measured in Ultra-Pure Air and Recovery from the Breath Collection System

Sample	Ethane	Pentane
Ultra-pure air, 4.0 1	21.2 ± 1.5	4.9 ± 1.2
Ultra-pure air, 4.0 1		
collected through breath collection system	22.6 ± 3.4	4.4 ± 0.0
Ultra-pure air, 4.0 1 + 82 pmol alkane standard[a]	108.6 ± 5.7	87.8 ± 4.0*
Ultra-pure air, 4.0 1 + 82 pmol alkane standard		
collected through breath collection system	104.4 ± 4.4	81.4 ± 2.9*

Note. Results are means ± SD ($n = 5$) and are expressed as pmol alkane.

[a] From alkane standard mixture (0.5 ppm ethane/0.5 ppm pentane).

* Pentane recoveries are significantly different ($P < 0.05$) by unpaired t test.

likely due to pentane from other sources, such as intestinal bacteria or intestinal contents (7).

In order to compare the amount of pentane produced by these other sources to the amount of pentane exhaled in breath, we compared the amount of ethane and pentane that evolved from rats in the live and dead states (Table 3). After the air-breath collections in the live state, rats were killed with carbon dioxide and then immediately placed back in the chamber for three more subsequent 20-min collections. The data in Table 3 show that ethane evolution rates decreased markedly (88%) after the rats were killed, while pentane evolution rates decreased slightly less (73%) after death. These data suggest that ethane evolves primarily

through breath, whereas as much as 27% of collected pentane may evolve from sources other than breath.

Theoretically, it would seem that isolating the rat's head would permit the collection of pentane derived exclusively from breath. However, we found low and fairly reproducible pentane excretion rates (i.e., comparable to reported rates from studies in which the head was isolated) for whole rats in the respiratory chamber as long as fecal matter was not present in the chamber. This gave us confidence in collecting total-body pentane by our method, which is easier, more practical, and less stressful to the animal than methods that isolate the head (4).
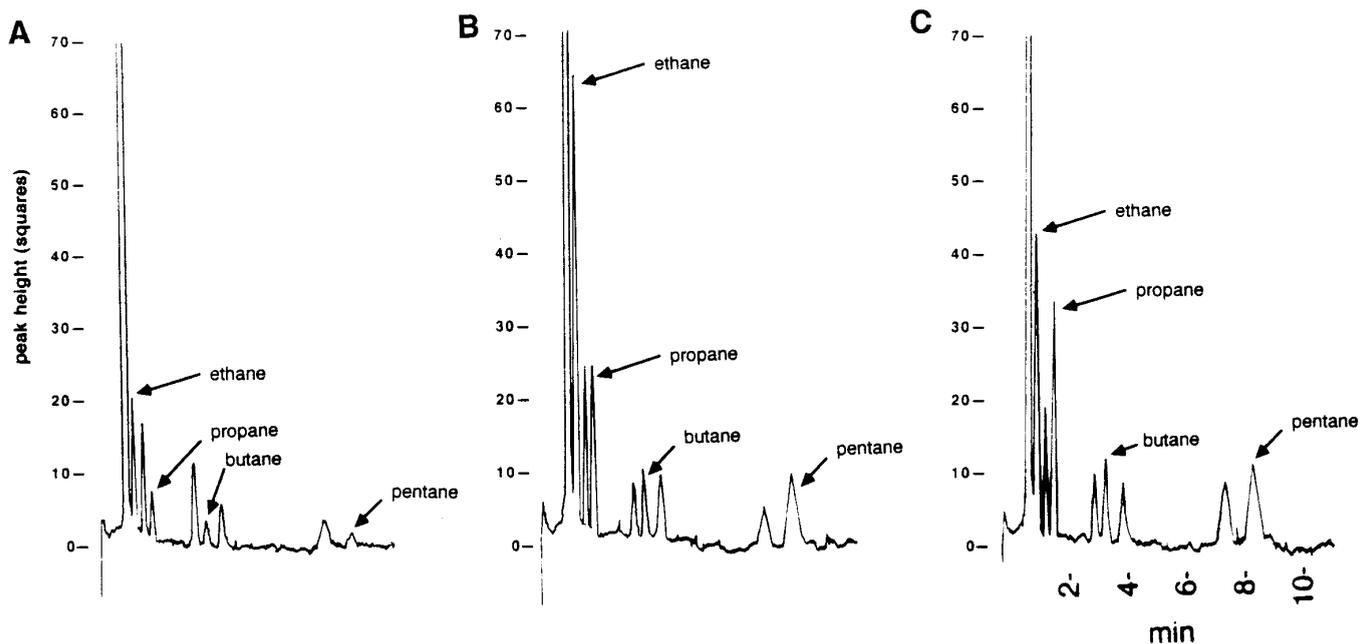
The origin of pentane exhaled in breath is not well

FIG. 4. GC chromatograms of (A) ultra-pure air, (B) an air-breath sample collected from a 215-g rat, (C) an ultra-pure air sample collected from respiratory chamber containing 0.68 g of fresh rat feces ($\frac{1}{3}$ of total fecal output from 1 rat). All samples (4 liters) were collected through the breath collection system over 20 min. Chromatograms represent the analysis of 5 ml of a total of 50 ml that was concentrated from the collected 4 liters.

TABLE 2

Inter-rat Variability and Day-to-Day Variability of Rat Breath Alkanes

	Day 1		Day 2		Day 3	
Sample	Ethane	Pentane	Ethane	Pentane	Ethane	Pentane
Rat # 1	1.02 ± 0.14	0.41 ± 0.04	0.94 ± 0.06	0.34 ± 0.10	0.96 ± 0.14	0.35 ± 0.06
Rat # 2	1.23 ± 0.15	0.49 ± 0.04	0.95 ± 0.25	0.52 ± 0.04	1.15 ± 0.16	0.34 ± 0.03
Rat # 3	1.17 ± 0.07	0.45 ± 0.04	1.15 ± 0.28	0.94 ± 0.04		
Rat # 4	1.19 ± 0.18	0.80 ± 0.04				

Note. Results are means ± SD ($n = 3$) and are expressed as pmol alkane/100 g body weight/min.

known. Some evidence strongly suggests that the majority of pentane in breath is not from membrane lipid peroxidation but from intestinal bacteria (7). Other investigators express concern that because pentane is highly soluble in viscera, muscle, and fat, any pentane that is exhaled in breath may represent a slowly diffusing tissue level of solubilized environmental and metabolic pentane (17). Overall, while the *in vivo* measurement of pentane may not exclusively indicate lipid peroxidation occuring in cell membranes, it may adequately reflect the degree of whole-body lipid peroxidation because pentane is derived from the major class of lipids in the body, the n-6 fatty acids.

As a marker of *in vivo* lipid peroxidation in the rat, ethane appears to be less affected by the problems noted above: it is relatively insoluble in tissues, it rapidly diffuses into the lungs, and it is metabolized very little in comparison to pentane (15). In addition, the collection and concentration of ethane is beset by fewer problems than is pentane. The recovery of ethane

TABLE 3

Evolution Rates of Alkanes from Rats
in the Live and Dead States[a]

	Ethane		Pentane	
Rat #	Alive[b]	Dead[c]	Alive[b]	Dead[c]
5	1.97 ± 0.21	0.19 ± 0.02	0.99 ± 0.29	0.18 ± 0.13
6	1.13 ± 0.20	0.07 ± 0.10	0.71 ± 0.19	0.26 ± 0.05
7	1.26 ± 0.21	0.24 ± 0.19	0.76 ± 0.13	0.19 ± 0.07

Note. Results are means ± SD ($n = 2–3$) and are expressed as pmol alkane/100 g body weight/min.

[a] Rats were killed with carbon dioxide and immediately placed in the respiratory chamber, which was then flushed for 25 min with ultra-pure air. Three 20-min dead-state collections were performed between 25 and 85 min after death.

[b] Alive-state ethane evolution rates are significantly different from ($P < 0.05$) alive-state pentane evolution rates by one-way ANOVA, using Scheffe's post hoc test.

[c] Ethane and pentane evolution rates in the dead state are significantly different from ($P < 0.05$) from those in the alive state by one-way ANOVA, using Scheffe's post hoc test.

through the breath collection system is more consistent than that of pentane (Table 1), and because ethane has a boiling point of $-89°C$, it will not condense on system components as may pentane.

Although ethane appears to be a more sensitive measure of *in vivo* lipid peroxidation, we were unable to effectively cryofocus it with a commonly used trapping technique that uses activated alumina cooled to $-130°C$ in an ethanol-liquid nitrogen bath (4, 16, 18). Other investigators report similar findings (9). With the present method, less than 20% of 90 pmol of ethane is cryofocused when the adsorbants are cooled to $-130°C$ instead of $-174°C$ (data not shown). The relatively large volumes of air-breath sample we concentrate (4 liters) and the fast rate at which the air-breath samples are passed through the adsorbants may also contribute to our inability to efficiently cryofocus ethane at $-130°C$. In any case, we demonstrate here that the lower temperature achieved with the use of liquid nitrogen ($-174°C$) is very efficient at cryofocusing essentially 100% of pentane and ethane. The use of liquid nitrogen to cool the adsorbants is also much less cumbersome and is less subject to temperature variations than a $-130°C$ ethanol–liquid nitrogen bath.

Even with using an open-flow respiratory chamber, the possibility exists that a portion of the alkanes evolved from the rat could be metabolized (especially pentane), provided that the evolved alkanes are not quickly cleared from the chamber into the gas sampling bag. In order to test this possibility, we collected 20 min air-breath samples from several rats after having added a 2 ml bolus of alkane standard (45 pmol ethane/ 45 pmol pentane) to the respiratory chamber. The ethane and pentane contents of these bags were compared to those in which the same amount of alkane standard was added not to the respiratory chamber, but directly to the collected air-breath samples in the bags. Identical results for ethane were obtained (data not shown) with each method of alkane addition, whereas pentane values were approximately 10% less when collected through the breath collection system containing the rat. These lower recoveries are probably not due to any

metabolism of pentane by the rat; instead, they most likely reflect the decreased mobility of pentane through the system, as was found in the validation experiments (Table 1). We conclude from these data that rats in the open-flow respiratory chamber do not metabolize to any significant degree the added ethane or pentane.

It has been reported that propane and butane are the predominant alkanes in rat feces, whereas ethane and pentane prevail in breath (13). We have found that rat feces, when in the respiratory chamber, exude significant amounts of ethane and pentane, in addition to propane and butane (Fig. 4C). Differences between the two studies may be due to differences in rat diets or in experimental conditions. In any case, we avoid the possibility of ethane and pentane contamination by fecal material in the respiratory chamber by ensuring that the rat defecates prior to the collection of air-breath samples. If fecal material is found in the chamber after collections, the collections should be repeated without feces.

Advantages of the Present Method

The main advantages of collecting and concentrating alkanes using the present method include the following: (i) The breath collection system is constructed from simple and inexpensive equipment; this allows the set-up of multiple collection systems and permits the simultaneous collection of breath samples from several rats. (ii) Respiratory chamber sizes can easily be varied to accomodate various sizes and species of small laboratory animals. (iii) The cryofocusing procedure, which uses liquid nitrogen at −174°C to cool the adsorbants, efficiently traps highly volatile ethane, even from large volumes of air and breath samples that are voided at a fast rate for cryofocusing. (iv) The flow of air through the open-flow respiratory chamber prevents the evolved alkanes from being metabolized by the rat before they are collected in the gas sampling bag. (v) Multiple breath samples from the same rat can be collected and stored for up to 2 weeks with only 10% loss of ethane (3). (vi) The entire process is relatively rapid (the flushing of the chamber, the collection, concen-

tration, and GC analysis of ethane and pentane requires 20, 20, 10, and 10 min, respectively) considering that multiple animals can be studied simultaneously.

REFERENCES

1. Dargel, R. (1992) *Exp. Tox. Path.* **44,** 169–181.
2. Frankel, E. M. (1983) *Prog. Lipid Res.* **22,** 1–33.
3. Kneepkens, C. M. F., Lepage, G., and Roy, C. C. (1994) *Free Radical Biol. Med.* **17,** 127–160.
4. Dillard, C. J., Dumelin, E. E., and Tappel, A. L. (1977) *Lipids* **12,** 109–114.
5. Habib, M. P., Eskelson, C., and Katz, M. A. (1988) *Am. Rev. Respir. Dis.* **137,** 341–344.
6. Schaeffer, H. J. (1989) *J. High Resol. Chromatogr.* **12,** 69–81.
7. Gelmont, D., Stein, R., and Mead, J. (1981) *Biochem. Biophys. Res. Commun.* **102,** 932–936.
8. Kneepkens, C. M. F., Ferreira, C., Lepage, G., and Roy, C. C. (1992) *Clin. Invest. Med.* **15,** 163–186.
9. Lawrence, G., and Cohen, G. (1984) *Methods Enzymol.* **105,** 305–311.
10. Reily, C. A., Cohen, G., and Lieberman, M. (1974) *Science* **183,** 208–210.
11. Hafeman, D. G., and Hoekstra, W. G. (1977) *J. Nutr.* **107,** 656–665.
12. Ogino, T., and Awai, M. (1988) *Biochim. Biophys. Acta* **958,** 388–395.
13. Kivits, G. A., Ganguli-Swarttouw, M. A., and Christ, E. J. (1981) *Biochim. Biophys. Acta* **665,** 559–570.
14. Daugherty, M. S., Ludden, T. M., and Burk, R. F. (1988) *Drug Metab. Dispos.* **16,** 666–671.
15. Frank, H., Hintze, T., Bimboes, D., and Remmer, H. (1980) *Toxicol. Appl. Pharmacol.* **56,** 337–344.
16. Sagai, M., and Ichinose, T. (1980) *Life Sci.* **27,** 731–738.
17. Pincemail, J., *et al.* (1990) *in* Free Radicals, Lipoproteins, and Membrane Lipids (Crastes de Paulet, A., *et al.,* Eds.), Plenum Press, New York.
18. Dumelin, E. E., and Tappel, A. L. (1977) *Lipids* **12,** 894–900.