papers and notes on methodology

Pitfalls in the use of breath pentane measurements to assess lipid peroxidation

John R. Springfield and Michael D. Levitt[1]

Research Service, Veterans Affairs Medical Center, One Veterans Drive, Minneapolis, MN 55417

Abstract Literature values for breath pentane, an indicator of lipid peroxidation, vary by 1,000-fold in healthy subjects. This report describes multiple technical artifacts that may explain this disparity. First, we found that a major component of human breath (probably isoprene) co-eluted with pentane on columns used by some investigators, resulting in erroneously high determinations. Second, despite washouts using pentane-free air, ambient pentane dissolved in body fat may result in breath concentrations many times greater than that due to endogenous production. True endogenous breath pentane may never have been accurately determined.—**Springfield, J. R., and M. D. Levitt.** Pitfalls in the use of breath pentane measurements to assess lipid peroxidation. *J. Lipid Res.* 1994. **35:** 1497–1504.

Supplementary key words calcium sulfate • lipid • glycerol • syringe • humans • rats

Tissue injury resulting from the action of oxygen free radicals is thought to play an important role in a variety of disease processes. One extensively studied form of free radical-mediated tissue injury is lipid peroxidation, a process that releases ethane and pentane from n–3 and n–6 polyunsaturated fatty acids, respectively (1). Ethane and pentane are excreted on the breath, and measurements of the pulmonary excretion of these gases have been widely used as quantitative indicators of free radical injury, in vivo.

Recently, a relatively simple technique was described to assay breath pentane (2), and application of this technique was said to demonstrate increased lipid peroxidation in patients with multiple sclerosis (3) and myocardial infarction (4). During our attempts to use this assay procedure, it became apparent that there were multiple pitfalls in the measurement of breath pentane. This report describes the complexities of breath pentane measurement and concludes that the enormous discrepancy (1000-fold) in breath pentane concentrations reported for normal adult subjects reflects several different technical artifacts.

MATERIALS AND METHODS

Collection of expired air

End alveolar breath samples were obtained from healthy human subjects using a commercial device that excludes the first 500 ml of expired air (dead space) and then collects the subsequent exhalation in a foil bag (GaSampler, Quintron, Milwaukee, WI). In studies with rats, the animals were housed in a closed system consisting of a two-piece Plexiglas® box, in which water served to seal an inner, rat-containing chamber from an outer chamber (see **Fig. 1**). Carbon dioxide was removed from the system by the presence of barium hydroxide (Baralyme®, Chemetron Medical Division, Allied Healthcare Products, Inc., St. Louis, MO), and pentane-free O_2 in a reservoir maintained at atmospheric pressure was drawn in as the pressure in the chamber fell due to the consumption of O_2 and removal of exhaled CO_2. The air in the chamber was circulated using a magnetic bar and stirrer. The gas volume of the chamber was calculated from the volume of the inner chamber minus the volume of the rats (calculated from their weights, with density assumed to be one) and their food and water supply. Measurements obtained after instillation of a known quantity of pentane into the empty chamber showed a leakage rate of $< 2\%$/day.

Desaturation and metabolism studies

To measure the rate of environmental pentane that was washed out of rats, three animals housed in an 18-liter chamber were exposed for 20 h to an atmosphere whose initial concentration was 2,000–4,000 ppm. At the end of this period, air in the chamber was sampled for pentane analysis. The rats were then blown with a hair dryer (to

[1]To whom correspondence should be addressed.

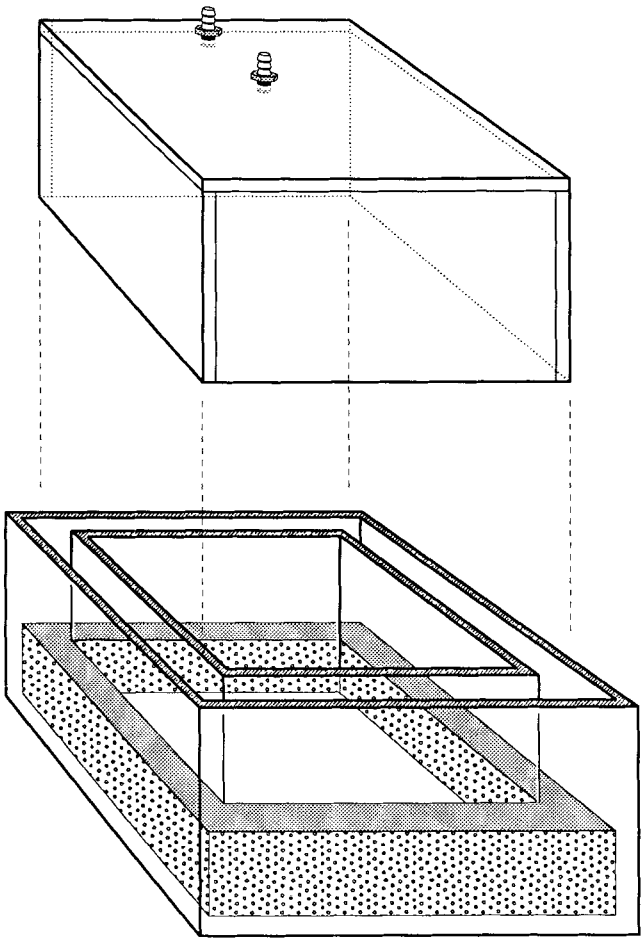

Fig. 1. Plexiglas metabolic chamber shown disassembled; top half fits between the walls of the inner and outer chambers of the lower half, resting in water (shown in shade) to create a sealed environment.

remove pentane trapped in hair) and transferred (approximately 15 sec elapsed in the transfer) to individual 3-liter chambers. Pentane present in the lungs at the time of transfer was estimated from the concentration of pentane in the initial chamber and an assumed lung volume of 7 ml (5). Measurements of pentane excretion were periodically obtained over 9 h (conventional rats) or 31 h (Zucker rats) by placing each rat in a 3-liter chamber for periods of 2–7 min. Background pentane and/or isoprene excreted by each set of rats was measured prior to their confinement in the high pentane environment, and was found to be insignificant in comparison to the high levels of pentane later excreted after their confinement.

Semilogarithmic plots of pentane excretion rate versus time showed an initial rapidly declining excretion followed, after about 3 h, by a slower, linear decline. The amount of pentane that would be excreted by the rat from this latter, slowly equilibrating pool from time t_0 (zero time) to the time at which the excretion rate would fall to 1 nmol/min was estimated as follows:

$$\text{Excretion} = \int_{t_0}^{t_f} A_f\, e^{-kt} = 1/k(A_0 - A_f)$$
$$\text{where } k = \ln_e (A_0/A_f)/t;$$

A_0 and A_f are, respectively, the rates of pentane excretion at zero time and the final time (t) when pentane excretion had fallen to 1 nmol/min.

Storage studies

The ability of various syringes to retain gaseous pentane was determined by filling 50-ml glass and plastic syringes (Becton Dickinson & Co., Franklin Lakes, NJ) with known concentrations of pentane in air. The syringes were sealed with plastic stopcocks, and the pentane concentration was followed over time.

Solubility studies

The relative solubility of pentane in air and lipid (corn oil), was determined using 50-ml glass syringes lubricated with glycerol. Twenty five ml of dilute pentane in air (300 ppm) was added to syringes containing 25 ml of lipid or blood. The syringes were initially vigorously shaken, and then incubated at 37°C on a rotating wheel. Samples of the gas were removed after 1 h and analyzed for pentane concentration.

Analyses

Analysis for pentane was performed on a Hewlett-Packard Model 5880A gas chromatograph using a flame ionization detector. Samples were injected via a 10-ml gas sampling valve onto a stainless steel column (6 ft × 1/8 in) packed with Chromosorb 102 (Hewlett-Packard), using argon carrier gas at a flow rate of 30 ml/min. Initial studies used a temperature gradient program similar to that described by Zarling and Clapper (2) for the resolution of three alkanes in addition to pentane: column temperature was held at 50°C for 1 min, increased by 30°C/min to 100°C (Zarling and Clapper (2) used 50°C/min), and the temperature was then increased by 15°C/min to 190°C and held at 190°C until pentane had completely eluted (approximately 12 min; see Fig. 2A). However, for the analysis of pentane in the absence of appreciable concentrations of other alkanes, a more convenient and equally effective separation was obtained using isothermal elution (140°C) at a higher flow rate (70 ml/min), and these conditions were used unless otherwise stated. The retention times and standard curves of pentane and isoprene were established using authentic standards (Fisher Scientific and Aldrich Chemical Company, respectively) prepared by enclosed vaporization of the liquid compounds. The lower limit of detection of pentane was approximately 40 pmol/l (or about 1 ppb).

In some studies, gases were directly injected into the gas sampling valve and then onto the column, and in

others the test gas was first dried via passage through a freshly prepared 60 × 6 mm precolumn consisting of Tygon® tubing packed with either 8 mesh anhydrous calcium sulfate (Drierite®, W.A. Hammond Drierite Co., Xenia, OH) or with 12 mesh anhydrous calcium chloride (Fischer Scientific Company). Other gas samples were incubated for 30–60 min in a 50-ml glass syringe containing an open, wide-mouthed vial holding potassium permanganate (2 ml, 0.1 M), to remove alkenes.

All data are expressed as means ± standard error of the mean (SEM).

RESULTS

Storage studies

The change in pentane concentration of standards stored in various fashions is shown in **Table 1.** A continuous and drastic fall in pentane concentration was observed with plastic syringes. The pentane concentration of gas samples stored in glass syringes lubricated with mineral oil fell by nearly 20% in the first minute and then remained relatively constant. In contrast, minimal loss of pentane was observed during storage in glass syringes lubricated with glycerol. All studies in this paper that required the use of syringes were carried out using glass syringes lubricated with glycerol.

Human breath studies

Fig. 2 shows the gas chromatographic retention times of authentic pentane and isoprene injected directly into the gas sampling valve. Both gases had similar retention times when analyzed with temperature programming (Fig. 2A and B) or isothermally at 140°C (Fig. 2C and D). Subsequent chromatographic studies were carried out isothermally at 140°C.

Fig. 3B shows a typical gas chromatographic tracing of expired air from a healthy subject with a peak whose retention time was virtually identical to that of authentic pentane (Fig. 3A). The apparent breath pentane concentration of normal subjects ranged from 1100 to 3700 pmol/l. However, when an aliquot of a breath sample was first slowly passed through a calcium sulfate precolumn, the previously noted "pentane" peak disappeared (Fig. 3C). A similar result was observed with a precolumn packed with calcium chloride (data not shown). In contrast, the concentration of the pentane standard was not altered by passage through the precolumn (Fig. 3D).

As shown in **Fig. 4A and 4B,** passage of an isoprene standard through the calcium sulfate precolumn removed most of the isoprene. Pretreatment of the isoprene standard (Fig. 4C) with aqueous potassium permanganate (0.01 M) removed virtually all of the isoprene (Fig. 4D) whereas similar treatment did not reduce the concentration of a pentane standard (Fig. 3E). Finally, treatment of an alveolar breath sample with potassium permanganate eliminated the apparent pentane peak (Fig. 3F and G).

TABLE 1. Retention of pentane in various syringes[a]

Syringe/Sealant[b]	% Retention over Time[c]		
	1 min	1 hour	18 hour
Plastic/rubber	96.6 ± 0.5	79.4 ± 0.6	38.9 ± 1.0
Glass/mineral oil	80.5 ± 2.7	76.8 ± 3.8	73.0 ± 4.0
Glass/glycerol	98.8 ± 0.2	97.5 ± 0.6	94.7 ± 0.9

[a] Concentration of pentane used was approximately 140 ppm.
[b] All syringes were 50–60 ml size from Becton Dickinson and Co., and were sealed using plastic stopcocks. Plastic syringes contained rubber seals, and the glass syringes were lubricated using mineral oil or glycerol, as indicated.
[c] Data are expressed as the mean ± SEM.

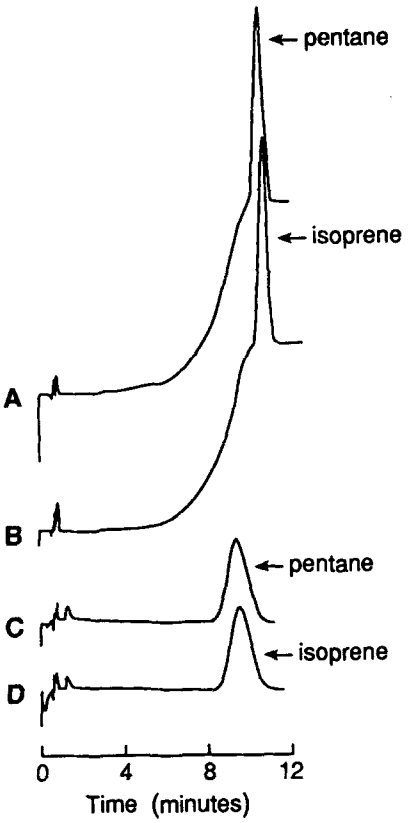

Fig. 2. Gas chromatograms (flame ionization detector) demonstrating the virtually identical elution profile of pentane (A) and isoprene (B) using temperature programming, or pentane (C) and isoprene (D) using isothermal elution; column and conditions described in Materials and Methods.

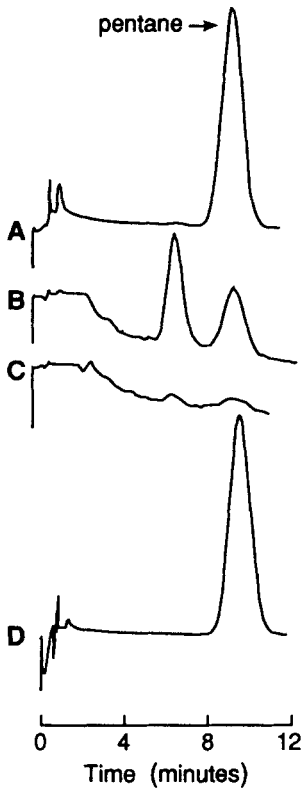

Fig. 3. Gas chromatograms (isothermal elution, as described in Materials and Methods) showing authentic pentane (A), and a sample of human breath before (B) and after (C) being passed through a precolumn packed with $CaSO_4$ (8 mesh, 60 × 6 mm); pentane was unaffected (D).

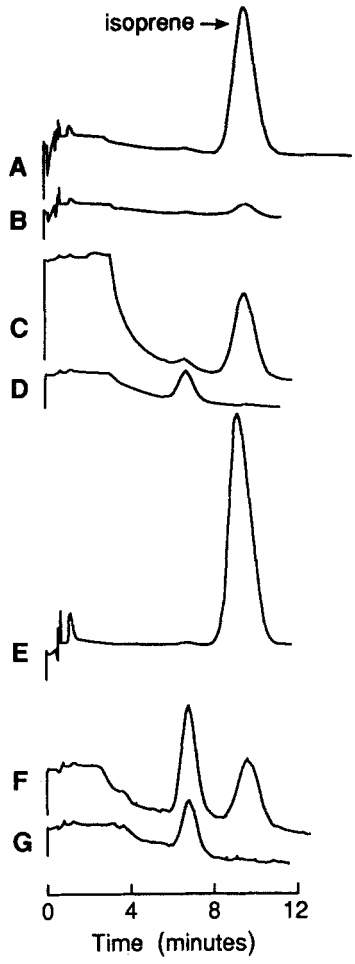

Fig. 4. Gas chromatograms (isothermal elution, as described in Materials and Methods) showing isoprene before (A) and after (B) passage through $CaSO_4$-packed precolumn (same column as described in legend to Fig. 3); isoprene before (C) and after (D) treatment with $KMnO_4$; pentane after treatment with $KMnO_4$ (E) (see 3A for untreated sample); and human breath before (F) and after (G) treatment with $KMnO_4$ (G). Samples treated with $KMnO_4$ were placed in 50 ml syringe containing 2 ml of aqueous 0.01 M $KMnO_4$ in a small wide-mouthed vial, and were left for 30-60 min.

Solubility studies

The solubilities at 37°C of pentane in lipid (corn oil) and in heparinized human blood relative to air were 53.2 ± 1.2 (n = 3) and 0.60 ± 0.05 (n = 4), respectively.

Desaturation and recovery studies

The rates of washout of environmental pentane from three conventional Sprague-Dawley rats (178 ± 8 g) and three genetically obese Zucker rats (258 ± 10 g) are shown in **Fig. 5.** A relatively rapid initial fall-off in pentane excretion was followed by a log linear fall-off that had an average half-time of 2.8 ± 0.2 h in the conventional rats and 8.5 ± 0.4 h in the Zucker rats. **Table 2** shows the environmental pentane estimated to have been taken up by the rat over 20 h, as well as that present in the lungs at the time of removal from the high pentane environment, and that recovered from slowly and from rapidly equilibrating tissues. An appreciable fraction of the pentane taken up by the rat was not subsequently recoverable. While most of the recovered pentane was dissolved in tissues (vs. in the lungs) of both types of rats, a much greater fraction was dissolved in the tissues of the Zucker rat.

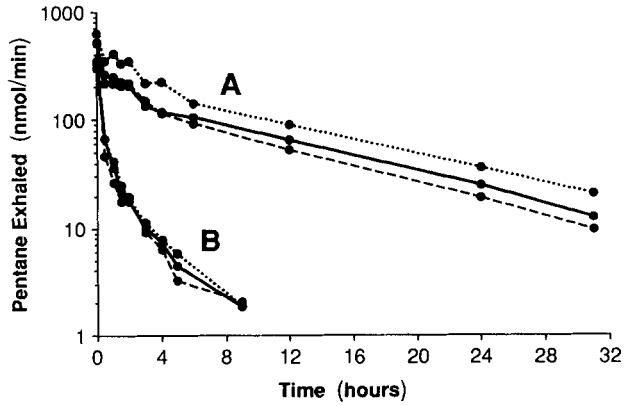

Fig. 5. Semilogarithmic plot of pentane excretion rate versus time of three Zucker rats (A) and three normal rats (B).

TABLE 2. Metabolism versus uptake of environmental pentane in the rat

Types of Rats	Uptake of Pentane over 20 h[a]	Pentane Recovery		
		Lungs[b]	Slowly Equilibrating Tissues (Fat)[c]	Rapidly Equilibrating Tissues[c]
	μmol/kg		*μmol/kg*	
Normal	900	0.62	24 ± 2	39 ± 4
Zucker (fat)	1020	0.57	520 ± 70	110 ± 20

[a]The values shown were estimated by dividing the drop in pentane concentration while the rats were confined together in closed chamber by the total weight of the rats.
[b]Values for the lung were calculated by assuming lung volumes of 7 ml per rat and a pentane concentration equivalent to that present at time of removal of rat from pentane-filled chamber and dividing by the average rat weight.
[c]Values for the slowly equilibrating (fat) tissues represent the means ± SEM, and were estimated from analysis of exhaled pentane at various times over 9 or 31 h for normal and Zucker rats, respectively, using the formula described in Materials and Methods, and dividing by the rat weight. Values for the rapidly equilibrating tissues were estimated by subtracting pentane excreted by slowly equilibrating tissues from the total pentane excreted, and dividing by the rat weight.

DISCUSSION

A striking feature of the literature concerning breath pentane concentrations of healthy adults is the greater than 1,000-fold (4.1–4900 pmol pentane/l) difference reported by various groups (see **Table 3**). This enormous discrepancy strongly suggests that technical errors influenced some of these measurements. Our studies suggest that these errors may take two forms: *1)* inaccurate measurement of breath pentane concentrations, and *2)* failure to differentiate between pentane of exogenous and endogenous origin.

TABLE 3. Measurements of breath pentane by various investigators

Number of Subjects	Washout	Pre-concentration of Breath Samples before GC Injection	Mean [Breath Pentane][a]	GC Column Packing	Ref.
	min		*pmol/l*		
5	10–15	yes	4.1	Porapak T	7
15	90	yes	12	Porapak T	8
3	10	yes	(18)	Porasil C	9
6	0	yes	~20	Activated Alumina	10
11	90	yes	(32)	Activated Alumina	11
11	60	yes	(37)	Activated Alumina	11
9	4	yes	(37)	Al_2O_3/KCl PLOT	12
15	0[b]	yes	79	Porapak T	8
19	4	yes	(81)	Porasil D	13
9	4	yes	(81)	Porasil D	14
10	4	yes	(89)	Porasil D	15
10	4	yes	(89)	Porasil D	16
10	4	yes	(106)	Porasil D	17
12	-	yes	134	Poraplot U	18
9	3	yes	~140[d]	Porasil D	19
15	-	no	590	Porasil C	20
6	-	yes	640	Porasil C	21
21	-	no	880	Porasil C	22
10	-	no	1710	Chromosorb 102	4
8	-	no	3700	Chromosorb 102	2
47	-	no	4020	Chromosorb 102	23
8	-	no	4900	Chromosorb 102	3

[a]Values in parentheses indicate results that were reported only in terms of pmol/kg/min instead of breath concentration, and were converted to pmol/l by assuming an average body weight of 70 kg and an average rate of alveolar ventilation of 5 l/min for healthy adults (6).
[b]No washout period preceded the experiment, but hydrocarbon-free air was breathed from zero time.
[c]Background pentane was subtracted.
[d]This estimated result was obtained from a graph in the referenced publication.

In our abortive attempt to measure breath pentane, we used the simple gas chromatographic technique of Zarling et al. (2) in which unconcentrated expired air is injected onto a Chromosorb 102 column. The highest reported breath pentane concentrations (1710–4900 pmol/l) have been obtained with this technique, and we found comparable "pentane" values for healthy subjects (1100–3700 pmol/l). However, when breath samples were dried via passage through calcium sulfate or calcium chloride precolumns, most of the "pentane" was eliminated (see Fig. 3). In contrast, the concentration of authentic pentane was not influenced by exposure to these drying agents, suggesting that the apparent pentane signal in breath was actually some co-eluting gas.

Examination of the literature (24) suggested that isoprene, a normal component of human breath, was a likely candidate to be the contaminating gas. Authentic isoprene and pentane had nearly identical retention times (Fig. 2), and virtually all isoprene was lost during passage through the calcium sulfate precolumn (Fig. 4). Compounds with double bonds, such as isoprene, are oxidized by potassium permanganate. Incubation with permanganate reduced the apparent pentane concentration in human breath to undetectable levels (< 40 pmol/l), but did not influence the concentration of authentic pentane. Thus, the high breath "pentane" concentrations reported by Zarling et al. (2) appear to be an artifact resulting from co-elution of another gas, and the results obtained by this technique need to be reevaluated. For example, elevated breath "pentane" concentrations were reported in patients with multiple sclerosis (3) and myocardial infarction (4). While these subjects presumably were excreting increased quantities of some gas (perhaps isoprene), the conclusion that excessive lipid peroxidation was occurring now seems unwarranted.

During preparation of our manuscript, Kohlmüller and Kochen (18) reported mass spectroscopic studies showing that isoprene in human breath does indeed co-elute with pentane on a number of commonly used gas chromatographic columns, including Porasil C, Tenax GC, activated Alumina, and GSQ/Poraplot Q, while Poraplot U appeared to give adequate separation. We found that this co-elution also occurs on columns packed with uncoated Carbopack B (6 ft, 200°C), as well as with the Chromosorb 102 column used in this study.

According to Kohlmüller and Kochen (18), the concentration ratio of isoprene:pentane in human breath is about 20:1. Thus, the use of inadequate columns by virtually all other investigators should have yielded breath pentane values 20 times higher than those reported by Kohlmüller and Kochen. However, some investigators (see Table 3) report human breath pentanes within and even well below the range (22–377 pmol/l) reported by Kohlmüller and Kochen (18). This paradoxical result may be explained by our observation that isoprene is avidly adsorbed by drying agents such as calcium sulfate or calcium chloride. All studies reporting values less than 700 pmol/l initially concentrated large volumes of expired air on precolumns maintained, in most instances, at very low temperatures. Although not always explicitly stated, presumably these large breath collections initially were passed through a column containing a drying agent to avoid deactivating the concentrating column and/or clogging the system with ice. As a result, contaminating isoprene may have been unwittingly removed by the drying agent. Kohlmüller and Kochen (18) did not treat breath samples with drying agents in conjunction with their cryotrapping apparatus.

Even if perfect analytical techniques had been used, it seems unlikely that previous studies have accurately distinguished pentane of exogenous origin from that derived from endogenous production. Surprisingly, most publications fail to report the atmospheric pentane concentration, a value that is critical to the interpretation of breath pentane measurements. If atmospheric pentane is low relative to the breath concentration, there is no need to eliminate the exogenous component. On the other hand, if ambient pentane is high relative to the breath pentane concentration resulting from endogenous production, extraordinary care is required to eliminate the exogenous component.

The best available data on atmospheric pentane is provided by environmental quality studies, a review (25) of which showed pentane concentrations in seven urban centers ranging from 12 to 38 ppbC (2.4–7.6 ppb), or about 110–340 pmol/l. These concentrations are appreciably greater than the breath pentane concentrations reported with prolonged breathing of pentane-free air (see Table 3). More direct evidence that environmental pentane greatly exceeds that resulting from endogenous production is the observation that breathing pentane-free air results in a precipitous fall in breath pentane concentration (8). Thus, accurate assessment of endogenous pentane production requires maneuvers to eliminate the atmospheric component. To this end, investigators have had subjects breathe pentane-free air for 3–90 min prior to the collection of the breath sample. As shown in Table 3, such washouts reduce the exogenous component, as evidenced by the trend toward lower breath pentanes for longer washout periods.

However, our studies in rats suggest that even a 90-min washout is inadequate. The pentane solubility (v/v) in lipid and blood is about 53 and 0.60 times that in air. Given this very high lipid solubility, one would predict that body fat should provide an enormous pentane sink that will turn over very slowly due to the low blood:lipid solubility of this gas and the low blood flow per gram of fat. These predictions were confirmed in studies of rats initially exposed to a high pentane environment for 20 h (see Fig. 5). After removal from this environment, an ini-

tial relatively rapid decline in pentane excretion was followed after about 3 h by a slow exponential decay that had a half-time of about 3 h in conventional rats and 8.5 h in congenitally obese Zucker rats. The initial rapid fall in excretion reflects loss of pentane from the lungs and rapidly equilibrating tissue pools. The prolonged, slowly declining phase of excretion presumably represents pentane dissolved in body fat. The ratio of the pentane recovered from body fat versus that in the lungs was calculated to be about 110 for the conventional rat (body fat: 7% (26)) and 1100 for the Zucker rat (body fat: 50% (27)) (see Table 2). Thus, while 90-min washouts would clear exogenous pentane from the lungs and rapidly equilibrating tissues, the vast preponderance of exogenous pentane still would remain in the slowly equilibrating body fat stores.

The above observations have important implications for human studies. Fat comprises about 28% and 39% of the body weight of normal weight adult males and females, respectively (28). Thus, the distribution of environmental pentane in fat versus the lungs should fall between that observed with conventional and Zucker rats, while in obese human subjects values observed in Zucker rats would be expected. Since humans have a much lower alveolar ventilation/kg and cardiac output/kg than do rats, pentane in human adipose tissue probably will washout even more slowly than is the case with the rat. Given the approximately 3–8.5 h half-times observed in rats, washout of most of the exogenous pentane from human subjects should require many hours of exposure to pentane-free air. Thus, even the longest washout periods (90 min) reported in human studies appear totally inadequate, and so it seems likely that the rate of excretion of endogenous pentane in humans has never been accurately measured.

While baseline pentane excretion probably is largely of exogenous origin, transient increases in "pentane" excretion observed after some perturbations appear to reflect true increases in endogenous production. Several studies of rats or rat tissue have demonstrated that after an oxidant stress, pentane release correlated with other markers of lipid peroxidation such as lipid-soluble fluorophores, thiobarbituric acid-reacting substances, and conjugated dienes, as well as with dietary and/or tissue vitamin E content (29). However, the possibility remains that some manipulation might increase the washout of pentane from fat stores via affects on the lipid content of blood, the rate of blood perfusing the adipose tissue, or the respiratory rate. Such factors need to be considered before isolated alterations in breath pentane excretion are confidently attributed to enhanced lipid peroxidation.

Several-fold increases of breath pentane over baseline levels observed with oxidative stresses have been interpreted as indicative of a several-fold increase in lipid peroxidation. However, as baseline excretion in all likelihood was predominantly exogenous pentane, the true increase in lipid peroxidation may be many-fold greater than was apparent from the percentage increase in breath pentane excretion. Thus, elevated breath pentane levels may be indicative of much greater increases in lipid peroxidation than heretofore recognized.

The previously reported metabolism of pentane (1) represents still another problem with the use of this gas as a quantitative indicator of lipid peroxidation. Our studies indicated that of the pentane taken up by the rat over 20 h, only about 2.4% (normal rat) or 21% (Zucker rat) was subsequently excreted, with the remainder apparently being catabolized. Thus, interpretation of even extremely accurate measurements of endogenous breath pentane could be confounded by inter-individual variations in pentane catabolism or alterations in the rate of catabolism in an individual.

A final pitfall in pentane measurements concerns the difficulty encountered in using syringes to handle and store this gas. Plastic syringes or glass syringes lubricated with mineral oil lost pentane at a rapid rate due to the enormous lipid solubility of this gas. Most reports do not discuss how the gas samples were handled although Snider et al. (11) used metal and glass surfaces in their apparatus, and Kohlmüller and Kochen (18) stored samples in stainless steel bombs. We found that pentane was lost at a relatively slow rate from glass syringes lubricated with glycerol, and the use of such syringes markedly simplifies experiments with this gas.

We are indebted to Dr. Eugene DeMaster for helpful advice. This work was supported in part by Veterans Affairs Merit Review Funds and National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases Grant 2 RO1 DK13309-22.

Manuscript received 11 August 1993 and in revised form 10 February 1994.

REFERENCES

1. Dumelin, E. E., and A. L. Tappel. 1977. Hydrocarbon gases produced during in vitro peroxidation of polyunsaturated fatty acids and decomposition of preformed hydroperoxides. *Lipids.* **12:** 894–900.
2. Zarling, E. J., and M. Clapper. 1987. Technique for gas-chromatographic measurement of volatile alkanes from single-breath samples. *Clin. Chem.* **33:** 140–141.
3. Toshniwal, P. K., and E. J. Zarling. 1992. Evidence for increased lipid peroxidation in multiple sclerosis. *Neurochem. Res.* **17:** 205–207.
4. Weitz, Z. W., A. J. Birnbaum, P. A. Sobotka, E. J. Zarling, and J. L. Skosey. 1991. High breath pentane concentrations during acute myocardial infarction. *Lancet.* **337:** 933–934.
5. Altman, P. L., and D. S. Dittmer. 1971. Respiration and Circulation. Federation of American Societies for Experimental Biology, Bethesda, Maryland. 111.
6. Altman, P. L., and D. S. Dittmer. 1971. Respiration and Circulation. Federation of American Societies for Experimental Biology, Bethesda, Maryland. 42.
7. Pincemail, J., G. Camus, A. Roesgen, E. Dreezen, Y. Bertrand, M. Lismonde, G. Deby-Dupont, and C. Deby. 1990.

Exercise induces pentane production and neutrophil activation in humans. Effect of propranolol. *Eur. J. Appl. Physiol.* **61:** 319–322.
8. Morita, S., M. T. Snider, and Y. Inada. 1986. Increased n-pentane excretion in humans: a consequence of pulmonary oxygen exposure. *Anesthesiology.* **64:** 730–733.
9. Wispe, J. R., E. F. Bell, and R. J. Roberts. 1985. Assessment of lipid peroxidation by measurements of expired ethane and pentane: influence of parenteral lipid infusion. *Pediatr. Res.* **19:** 374–379.
10. Dillard, C. J., R. E. Litov, W. M. Savin, E. E. Dumelin, and A. L. Tappel. 1978. Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *J. Appl. Physiol.* **45:** 927–932.
11. Snider, M. T., P. O. Balke, K. E. Oerter, N. A. Francalancia, K. A. Pasko, M. E. Robbins, G. S. Gerhard, and R. B. Richard. 1985. Methods for measuring lipid peroxidation products in the breath of man. *Life Chem. Rep.* **3:** 168–173.
12. Kneepkens, C. M. F., C. Ferreira, G. Lepage, and C. C. Roy. 1992. The hydrocarbon breath test in the study of lipid peroxidation: principles and practice. *Clin. Invest. Med.* **15:** 163–186.
13. Hoshino, E., R. Shariff, A. Van Gossum, J. P. Allard, C. Pichard, R. Kurian, and K. N. Jeejebhoy. 1990. Vitamin E suppresses increased lipid peroxidation in cigarette smokers. *J. Parenter. Enteral. Nutr.* **14:** 300–305.
14. Van Gossum, A., J. De Cuyper, H. Ooms, M. Cremer, and K. N. Jeejeebhoy. 1992. Assessment of lipid peroxidation in humans by breath pentane output measurement. *Acta Gastroenterol. Belg.* **55:** 245–248.
15. Lemoyne, M., A. Van Gossum, R. Kurian, M. Ostro, J. Axler, and K. N. Jeejeebhoy. 1987. Breath pentane analysis as an index of lipid peroxidation: a functional test of vitamin E status. *Am. J. Clin. Nutr.* **46:** 267–72.
16. Van Gossum, A., S. Riaz, M. Lemoyne, R. Kurian, and K. Jeejeebhoy. 1988. Increased lipid peroxidation after lipid infusion as measured by breath pentane output. *Am. J. Clin. Nutr.* **48:** 1394–1399.
17. Kohlmüller, D., and W. Kochen. 1993. Is n-pentane really an index of lipid peroxidation in humans and animals? A methodological reevaluation. *Anal. Biochem.* **210:** 268–276.
18. Van Gossum, A., R. Kurian, J. Whitwell, and K. N. Jeejeebhoy. 1988. Decrease in lipid peroxidation measured by breath pentane output in normals after oral supplementation with vitamin E. *Clin. Nutr.* **7:** 53–57.
19. Kanter, M. M., L. A. Nolte, and J. O. Holloszy. 1993. Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and postexercise. *J. Appl. Physiol.* **74:** 965–969.
20. Moscarella, S., L. Caramelli, P. F. Mannaioni, and P. Genetilini. 1984. Effect of alcoholic cirrhosis on ethane and pentane levels in breath. *Boll. Soc. Ital. Biol. Sper.* **60:** 529–533.
21. Hempel, V., R. May, H. Frank, H. Remmer, and U. Köster. 1980. Isobutene formation during halothane anaesthesia in man. *Br. J. Anaesth.* **52:** 989–992.
22. Moscarella, S., G. Laffi, G. Buzzelli, R. Mazzanti, L. Caramelli, and P. Gentilini. 1984. Expired hydrocarbons in patients with chronic liver disease. *Hepatogastroenterology.* **31:** 60–63.
23. Zarling, E. J., S. Mobarhan, P. Bowen, and S. Kamath. 1993. Pulmonary pentane excretion increases with age in healthy subjects. *Mech. Ageing Dev.* **67:** 141–147.
24. DeMaster, E. G., and H. T. Nagasawa. 1978. Isoprene, an endogenous constituent of human alveolar air with a diurnal pattern of excretion. *Life Sci.* **22:** 91–98.
25. Sexton, K., and H. Westberg. 1984. Nonmethane hydrocarbon composition of urban and rural atmospheres. *Atmos. Environ.* **18:** 1125–1132.
26. Bray, G. A. 1976. The Obese Patient. W. B. Saunders Company, Philadelphia, PA. 156–157.
27. Larkin, L. M., B. J. Moore, J. S. Stern, and B. A. Horowitz. 1991. Effect of photoperiod on body weight and food intake of obese and lean Zucker rats. *Life Sci.* **49:** 735–745.
28. Cohn, S. H., K. J. Ellis, D. Vartsky, A. Sawitsky, W. Gartenhaus, S. Yasumura, and A. N. Vaswani. 1981. Comparison of methods of estimating body fat in normal subjects and cancer patients. *Am. J. Clin. Nutr.* **34:** 2839–2847.
29. Dillard, C. J., and A. L. Tappel. 1989. Lipid peroxidation products in biological tissues. *Free Radical. Biol. Med.* **7:** 193–196.