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COMMENTARY

EXHALED ALKANES AS INDICES OF *IN VIVO* LIPID PEROXIDATION

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Lipid peroxidation is suspected of being involved in a number of pathological processes. Proof of this has been very difficult to obtain, however. A major problem has been the lack of a technique to quantitate precisely rates of lipid peroxidation in animals and thus to assess accurately differences between experimental groups. In simple systems such as isolated liver microsomes, quantitation of lipid peroxidation can be achieved by measuring the disappearance of a substrate, such as polyunsaturated fatty acid, or the appearance of a polyunsaturated fatty acid breakdown product, such as malondialdehyde. As experimental systems become more complex, these measurements lose their precision. Cells can replace polyunsaturated fatty acids that have been peroxidized and can metabolize malondialdehyde. Thus, measurements of these substances may not yield accurate estimates of lipid peroxidation rates in experimental preparations containing functioning cells. This includes, of course, living animals.

An ideal index of lipid peroxidation would be a substance that is produced in a fixed stoichiometry with peroxidized polyunsaturated fatty acid and that is not metabolized or otherwise lost from the compartment in which it is formed and measured. When ethane was reported in 1974 to be produced by *in vivo* lipid peroxidation [1], it was thought to be such an ideal index. It and the related alkane, pentane, have been used extensively to assess lipid peroxidation in living animals and in simpler systems. However, in recent years it has become apparent that ethane and pentane are not ideal indices of lipid peroxidation. This commentary focuses on two processes which affect the utility of these alkanes in the measurement of lipid peroxidation: oxygen-induced variation in their formation stoichiometry and metabolism of the alkanes. In concordance with most of the literature, this commentary uses pentane to refer specifically to *n*-pentane.

Oxygen effects on the stoichiometry of alkane formation

The alkanes ethane and pentane are generated from ω-3 and ω-6 polyunsaturated fatty acids respectively (see Ref. 2 for review of mechanisms). The final steps by which ethane is generated are illustrated in equations 1 and 2:

$$H_3C{-}CH_2{-}\underset{\displaystyle O\cdot}{\underset{|}{CH}}{-}R \rightarrow H_3C{-}CH_2\cdot + O{=}CH{-}R \quad (1)$$

$$H_3C{-}CH_2\cdot + R'H \rightarrow H_3C{-}CH_3 + R'\cdot \quad (2)$$

An ethyl radical arises from β-scission of an alkoxy radical (equation 1). There follows a hydrogen abstraction by the ethyl radical to form ethane (equation 2). In the case of pentane formation, a pentyl radical results from β-scission.

In 1982, Cohen [3] reported some observations suggesting that oxygen concentration could affect the yield of alkanes arising from lipid peroxidation. He confirmed that increasing the oxygen tension in peroxidizing tissue homogenates caused an increase in lipid peroxidation products which reacted with thiobarbituric acid (malondialdehyde). At the same time, however, he noted that alkanes fell with increasing oxygen tension. Cohen suggested that oxygen reacted with alkyl radicals more rapidly than hydrogen abstraction could occur (equation 3):

$$H_3C{-}CH_2\cdot + O_2 \rightarrow H_3C{-}CH_2OO\cdot \quad (3)$$

This would lead to oxygen-containing products rather than to ethane. There is evidence that such products are generated by lipid peroxidation [2].

The oxygen effect on alkanes was examined in more detail by other groups. Kostrucha and Kappus [4] compared alkane formation with malondialdehyde formation in a rat liver microsomal system. They reported an inverse relationship between these two indices of lipid peroxidation as oxygen tension varied. Alkane formation fell and malondialdehyde formation rose when oxygen tension was increased. They argued that because malondialdehyde formation required addition of two oxygen molecules to the lipid and alkane formation required only one, conditions of high oxygen tension favored the pathway to malondialdehyde. This explanation implies

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that the molar yield of malondialdehyde from lipid peroxidation increases and that of alkanes decreases with increments in oxygen tension.

Another study of peroxidizing rat liver microsomes was carried out by Reiter and Burk [5]. They measured loss of polyunsaturated fatty acids as a frame of reference for other measures of lipid peroxidation. Oxygen concentrations from below 1% to 100% were studied. With increases in oxygen concentration polyunsaturated fatty acid loss increased, signifying increasing lipid peroxidation. The yield of malondialdehyde from polyunsaturated fatty acid did not vary with oxygen tension. In contrast, alkane yield fell strikingly with increasing oxygen concentrations. Below 5% oxygen, pentane was formed from ω-6 polyunsaturated fatty acids at a molar yield of ~0.3% when an iron-ascorbate radical-generating system was employed. The yield fell to 0.05% at 21% oxygen and to 0.003% at 100% oxygen. Quantitatively similar effects were found on ethane formation. Thus, with one version of the iron-ascorbate lipid peroxidation system, alkane formation mirrored polyunsaturated fatty acid loss below 5% oxygen but not at higher oxygen tensions. The molar yield of alkane found at 100% oxygen was only 1% that found at 5% oxygen.

When a CCl_4 radical-generating system was used, the molar yield of alkane to polyunsaturated fatty acid loss below 5% oxygen varied inversely with oxygen concentration, unlike the findings with the iron-ascorbate system. A potential explanation for this is that the iron-ascorbate system consumes enough oxygen in the initial formation of radicals that it decreases oxygen available (when atmospheric oxygen is 5% or lower) to react with alkyl radicals (equation 3), thus allowing them to perform hydrogen abstraction. The CCl_4 system generates radicals at a lower rate and should not consume as much oxygen. Thus, the microenvironment would contain oxygen which could compete with hydrogen abstraction. This suggests that at low oxygen concentrations characteristics of the experimental system employed, such as the nature and concentration of free radicals, will influence the yield of alkane.

These findings suggest the following schemes:

$$\text{polyunsaturated fatty acids} + O_2 \xrightarrow[\text{peroxidation}]{\text{lipid}} \text{intermediates} \qquad (4)$$

$$\text{malondialdehyde} \xleftarrow[\text{effect}]{\text{no } O_2} \text{intermediates} \xrightarrow[\text{inhibits}]{O_2} \text{alkanes} \qquad (5)$$

This scheme indicates that lipid peroxidation requires oxygen and occurs at a rate dependent on oxygen concentration (equation 4). This has been amply demonstrated in the past [6] and was confirmed in the study of Reiter and Burk [5]. Malondialdehyde formation is little affected by oxygen concentration in the system tested [5] and thus is a good index of lipid peroxidation regardless of the oxygen tension (equation 5). Alkane formation from intermediates of lipid peroxidation is highly oxygen sensitive (equation 5) and consequently is useful as an index of lipid peroxidation only when oxygen concentration is held constant. Moreover, alkane formation is more efficient at low oxygen concentrations and will be a more sensitive index in systems with low oxygen tensions. The effect of oxygen concentration on other products of lipid peroxidation remains to be assessed.

Metabolism of alkanes

In 1970 Frommer *et al.* [7] demonstrated that pentane was metabolized by rat liver microsomes. Subsequently, other investigators have shown that both pentane and ethane are metabolized *in vivo* by rats, mice, and humans [8–14]. One study conclusively demonstrated metabolism by showing that pentane and ethane are metabolized to carbon dioxide [14]. Following introduction of a bolus dose of [^{14}C]pentane or [^{14}C]ethane into a closed chamber containing a rat, 50 and 20%, respectively, of the dose were excreted in breath as $^{14}CO_2$ over an 8-hr period. Other studies have shown that pentane can be metabolized in *in vitro* systems used to assess lipid peroxidation [9, 10].

Frank *et al.* [8] proposed that the rate-limiting step in pentane metabolism is oxidation by the ethanol-induced cytochrome P-450. Terelius and Ingleman-Sundberg [15] confirmed the role of this form of cytochrome P-450 in the *in vitro* metabolism of pentane by hepatic microsomes and cytochrome P-450 preparations from rats and rabbits. They found pentane to be an extremely good substrate for the ethanol-inducible form of cytochrome P-450.

Recently, Allerheiligen *et al.* [16] described the kinetics of pentane in the intact rat following its administration in a closed chamber. They found that the metabolic clearance of pentane relative to whole blood pentane concentration was approximately 140 ml of blood/(min·kg) or about 50% of the cardiac output. This finding strongly implies that pentane is cleared by organs in addition to the liver and the intrinsic ability of eliminating organs to metabolize pentane (intrinsic clearance) is high. If hepatic extraction of pentane approached unity, the maximum hepatic clearance value would be about 70 ml/min·kg) or 25% of cardiac output. Therefore, metabolism by other organs appears to contribute to pentane elimination. The kidney and brain received about 25 and 15% of cardiac output, respectively, and, if their extraction ratios were moderate to high (0.3–1), these organs and the liver could account for the observed clearance value. In addition, a metabolic extraction ratio of as little as 0.1 for the lung could account for an organ clearance of 25–30 ml(min·kg) since it receives virtually all of the cardiac output. Finally, the high pentane clearance could be explained, in part, by the presence of a slowly equilibriating region of the body, such as adipose tissue, that acted as a sink over the 8-hr period of the experiment for pentane presented either by the blood or by diffusion across the skin. The probability of this latter explanation being correct is diminished by the extensive metabolism of pentane to CO_2 during the same time period [14] and the ability of carbon tetrachloride to block pentane clearance almost completely [11]. Ethanol and 4-methylpyrazole, which inhibit cytochrome P-450, also decrease pentane clearance [11, 16].

Ethane is metabolized much more slowly than pentane [9, 14], and this makes precise estimation of its clearance value more difficult. Unpublished studies based on a 16-hr sampling time in the closed chamber system used for pentane [16] indicate a clearance for ethane from blood of approximately 75 ml/(min·kg) (Allerheiligen SRB, Daugherty S, Ludden TM and Burk RF, unpublished results). In addition, the blood-to-air partition coefficient for ethane at 37° is about 0.1, much lower than the pentane value of 0.42 [16]. The combination of lower clearance and lower partition coefficient yields a much lower overall elimination rate for ethane than for pentane in the rat [14].

Excretion of alkanes in the breath requires that they diffuse from the site of formation into the blood and then be transported to the lung. Metabolism of the alkane could occur in the tissue where it is formed or in other tissues where it could be extracted from the blood. It seems highly likely that alkane formation and metabolism occur in the same tissue in some instances. In such a situation, the intrinsic ability of the tissue to metabolize the alkanes becomes very important. The relative rate of spillover of alkane from the site of production into the blood is a function of the rate of diffusion out of the cell versus the rate of metabolism. If the rate of diffusion out of the cell is constant, then changes in rate of metabolism can produce significant changes in the rate of spillover, particularly if the rate of metabolism is high. For example, if the ratio of spillover to production under basal conditions is only 0.1, then a complete inhibition of metabolism would yield a ratio of 1. This would cause a 9-fold increase in spillover rate, which could be interpreted as an increase in alkane formation. Results of the kinetic studies of pentane cited above, and to a lesser extent studies with ethane, indicate that the intrinsic clearance of these compounds may be high by one or more organs. Metabolism in those organs could complicate the use of alkanes to assess lipid peroxidation in them. This is almost certain to occur in the liver.

Metabolism in tissues other than the tissues of origin could affect alkane exhalation. Simulations based on a steady-state physiologic model that accounted for the architecture of the circulatory system have been carried out to estimate such an effect. These simulations predict only a moderate effect [17]. For example, the ratio of alkane exhaled to that leaving the tissue where it was formed could increase by 5–10% for ethane and by 20% for pentane if metabolic clearances were reduced to zero. This is because the observed clearances and partition coefficients in combination with typical values for alveolar ventilation rate, cardiac output, and blood volume yield ratios that are already close to 1. Alkanes released into venous blood other than that of the gastrointestinal tract must pass through the lung prior to reaching other organs of elimination. The low blood-to-air partition coefficients of ethane and pentane favor excretion into breath rather than recirculation. Thus, it seems that metabolism in the tissue of origin, which is a first-pass effect, may be more important than metabolism elsewhere in the body in modifying alkane exhalation rates.

One group has measured metabolism rates of alkanes administered in a closed system and has used the results to calculate formation rates from exhalation rates [13]. When this method is employed, it should be kept in mind that it cannot fully correct for the first-pass metabolism in the tissue of formation. Moreover, moderate changes in intrinsic clearance may be difficult to detect following exogenous administration of the alkane because clearance appears to be flow dependent. Such changes in intrinsic clearance could have major effects on metabolism in the tissue of formation.

At present there is no foolproof method to determine *in vivo* alkane formation rates. Measuring metabolism rates may allow improved estimates. Based on the information available, we conclude that the use of ethane is less likely to yield undetectable artifacts than is the use of pentane.

Conclusions

The unique properties of alkanes make them useful in measuring lipid peroxidation *in vivo*. However, several factors besides the rate of lipid peroxidation contribute to the rate of alkane exhalation. These factors must be considered in the interpretation of alkane exhalation rates.

The oxygen concentration at the site of alkane formation can affect the molar yield of these compounds. A 100-fold drop in molar yield has been demonstrated to accompany a rise in oxygen concentration from 5 to 100% [5]. The implication of this observation is that tissues with high oxygen concentrations will produce smaller amounts of alkanes than poorly-oxygenated tissues when they undergo lipid peroxidation. The centrilobular region of the liver has a very low oxygen concentration, and lipid peroxidation occurring in this region can be sensitively detected by exhaled alkanes [1]. Alkanes have been poor indices of free-radical injury with presumable lipid peroxidation in well-oxygenated organs such as the lung [18, 19]. Thus, the same rate of alkane exhalation could represent a large amount of lipid peroxidation occurring in a well-oxygenated tissue or a small amount of lipid peroxidation occurring in a poorly-oxygenated tissue. Because it is not possible to determine the origin of exhaled alkane, this severely limits the use of exhaled alkanes to implicate lipid peroxidation in specific pathologies.

Metabolism of alkanes occurs and is more rapid for pentane than for ethane. Inhibitors of metabolism such as ethanol or injury to tissues of metabolism can cause increased exhalation of alkanes. This can lead to a false conclusion that lipid peroxidation is occurring. There is evidence that alkane metabolism does not occur in all tissues of the body. Alkanes that arise in tissues which can metabolize them are subject to a first-pass effect and are probably metabolized to a greater extent than alkanes that arise in tissues which cannot metabolize them. Thus, metabolism of alkanes in the body is nonuniform, and efforts to correct for metabolism by measuring disappearance of inhaled alkanes may not yield accurate correction factors.

In spite of these limitations, the occurrence of lipid peroxidation can be inferred when very high rates of alkane exhalation are demonstrated. Caution should be exercised, however, in interpreting smaller

increases (on the order of several-fold) as evidence of lipid peroxidation and in using alkane exhalation rates to quantitate *in vivo* lipid peroxidation.

The effect of oxygen concentration on formation stoichiometry is similar for ethane and pentane so the choice of alkane cannot minimize this problem. However, pentane is more rapidly metabolized *in vivo* than is ethane and for this reason exhaled ethane is likely to reflect formation rate more closely than exhaled pentane. Based on these considerations, exhaled ethane would appear to be a better index of *in vivo* lipid peroxidation than exhaled pentane.

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