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IS PENTANE A NORMAL CONSTITUENT OF HUMAN BREATH?

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Breath analysis is a non-invasive method for investigation of the volatile compounds produced by humans. Pentane has often been taken as an indicator of lipid peroxidation. Our purpose in this study was to determine its normal concentration in the breath of healthy humans. Using a specific and sensitive gas chromatography-mass spectrometry technique pentane concentrations in breath were lower than 10 pmoles/l. The high levels of pentane found by some authors in healthy humans were probably due to the coelution of pentane with isoprene, a volatile hydrocarbon present in human breath.

KEY WORDS: Lipid peroxidation, breath, human, pentane, isoprene.

INTRODUCTION

Free oxygen radicals produced during normal and abnormal metabolism[1,2] could damage DNA proteins and lipids[2,3]. The peroxidation of polyunsaturated fatty acids is often regarded as one of the primary mechanisms of tissue damage.

Peroxidation of n-3 and n-6 fatty acid families results in the formation of ethane and pentane, respectively. Ethane production, in the liver of mice treated by CCl_4, is correlated with lipid peroxidation of the n-3 fatty acid family[4]. Pentane exhalation is higher in vitamin E-deficient rats fed with corn oil which is rich in linoleic acid (n-6 fatty acid family) than in vitamin E-supplemented rats[5,6]. Measurement of exhaled alkanes, especially ethane and pentane, has been used as a non-invasive technique for monitoring human lipid peroxidation[7-18].

Several gas chromatographic methods for the determination of pentane have been described during the last years[7-18]. But problems, linked to coelution of volatile compounds and to contamination of the air breathed by the subjects, may occur.

To measure unequivocally pentane in human breath, we have used a thermodesorption unit coupled to a gas chromatograph and a mass spectrometer and we have compared our results to those in the literature.

MATERIALS AND METHODS

We studied the breath of 15 healthy adult volunteers of the staff laboratory (8 women and 7 men, aged 22 to 55 years, mean age = 38,3 ± 10,7 years). Much care was taken for sampling expired air: the volunteers were asked to stay in the same room for 30 min before the breath collection in order to equilibrate with room volatile contaminants. Several breath samples of each volunteer were collected during the same day. The subjects were instructed to inhale moderately and then

to exhale as much as possible in a 1 l teflon bag. During this period, 1 l of indoor air was drawn up near each subject with a sampling pump (alpha 1 Dupont, Arelco, Fontenay sous Bois, France) and concentrated on an absorbent tube. The adsorbent tube was a silanized glass tube (11,5 × 0,4 cm I.D. Supelco, St Germain en Laye, France) packed with tenax (Chrompack, Les Ulis, France). Before analysis, one litre of expired air was transferred for concentration on these tenax tubes with the sampling pump. Then the tubes were thermally desorbed at 300°C during 2 min using a thermal desorption unit (Supelco) connected to the gas chromatograph.

Pentane was analyzed by gas chromatography (Model 6000 Varian, Les Ulis, France) using a 30-m capillary column (Poraplot U 0,32 mm I.D., Chrompack) connected to a mass spectrometer detector (ion trap detector 800 Finnigan, Orsay, France).

Standard pentane concentrations were obtained as follows: 5 μl of liquid pentane was measured by a syringe and introduced through a teflon-faced septum into a 25 ml vial. Pentane was rapidly vaporized at ambient temperature and after 5 min for equilibration 10 μl of pentane vapour phase was taken via an air-tight syringe and introduced into another 25 ml vial. For quantification 20 to 200 μl of vapour phase was injected into the gas chromatograph.

RESULTS

Human expired air contains numerous organic volatile compounds, some are from endogeneous origin, others are contaminants present in ambient air. In this study, the only compound of interest was pentane and the method was optimized for its determination. Different chromatographic columns were tested to avoid interferences between pentane and other organic volatile components. Pentane, as other saturated aliphatic hydrocarbons, does not have a well characterized mass spectrum. The main important fragment is $m/z = 41$ and the molecular ion is of very low intensity. Pentane was detected using its retention time and the reconstructed ion chromatogram at $m/z = 41$.

As the detection limit of a compound depends on the limit of instrument sensitivity and on chromatographic interferences from the sample matrix, an area of 1000 in arbitrary units was estimated to be the detection limit of pentane on the reconstructed ion chromatogram at $m/z = 41$. This correspond to a limit of 10 pmole/l for 1 l of air sample.

The concentrations of pentane found in expired air ranged from 10 to 50 pmoles/l, but its concentration in indoor air was similar (Table I). Thus, using a specific and sensitive method under our experimental conditions, we were not able to detect significant exhalation of pentane by healthy volunteers.

DISCUSSION

For several years we have been studying organic volatile compounds in human breath samples[19,20] and in spite of a large number of analysis we have never detected pentane. In our previous studies we used only 50 ml of breath, but in this study the analysis was performed after concentrating 1 l of expired air on adsorbent and by taking a maximum of care for the sampling of breath.

TABLE I
Concentration of pentane in human brath, analytical chromatographic columns and detectors used by the different authors

AUTHORS	ANALYTICAL COLUMN AND DETECTOR	PENTANE CONCENTRATION pmoles/l
DILLARD (1978)	Activated Alumina FID	10
HEMPEL (1980)	Porasil C FID	470 ± 30
MOSCARELLA (1984)	Porasil C FID	880
WISPE (1984)	Porasil C FID	200
WADE (1984)	Porasil C FID	120 ± 50
MORITA (1986)	Porapak T FID	200
PINCEMAIL (1987)	Porapak T FID	5 to 140
ZARLING (1987)	Chromosorb 102 FID	3 700
ZARLING (1988)	Chromosorb 102 FID	370 – 15 000
SHARIFF (1988)	Porasil D	100
SINON CHNASS (1988)	Not specified	40 000
HOTZ (1987)	Porasil C FID	breath = ambient
VAN GOSSUM (1987-88)	Porasil D FID	100
WEITZ (1990)	Chromosorb 102 FID	170
MASSIAS (1991)	Carbopack B FID	360
OUR RESULTS	Poraplot U MS	breath = ambient

In the literature many data on the levels of pentane in human air exist but there is a great discrepancy between the results, as shown in Table I. For easier comparison, the different results were converted into the same unit (pmoles/l) . The range is large, going from some pmoles/l to some thousands pmoles/l of expired air.

Table I shows that the separations of compounds are often done using non capillary columns and the detection obtained only by flame ionization which is a sensitive method for hydrocarbons, but less specific than mass spectrometry. We have tested different chromatographic columns, particularly those used by authors who found high pentane levels in breath, and we have observed that, on chromosorb 102 and on Carbopack B, pentane coelutes with isoprene, another volatile hydrocarbon. Isoprene is the main endogeneous volatile hydrocarbon in human expired air. Its presence has been described by several authors[21-25] and we have shown[20] that its concentration could depend on the state of sleep or wakefulness. The possible interference between pentane and isoprene, or other volatile compounds, cannot be detected using a flame ionization detector. Since the mass spectra of pentane and isoprene are very different (Figure 1) the use of a capillary column connected to a mass spectrometer avoids the ambiguity. Thus, the high levels of pentane found by some authors in breath from healthy subjects are probably the consequence of isoprene interference. Some authors have described also high breath pentane concentrations during rheumatoid arthritis[26] or acute myocardial infarction[27], but they cannot know if they measured an increase in pentane or in isoprene.

The limit of our technique is 10 pmoles/l of pentane in expired air and it seems difficult to lower this limit because the air breathed by the subjects contains always trace of pentane. Even the indoor air of the cleanest room is polluted with volatile chemicals generated from building materials and furnishings. The sampling of several liters of air will increase the risks of contamination, unless if the subjects stay in and breathe especially purified air.

Our results are in agreement with those of some authors. Seeger[28] using a highly

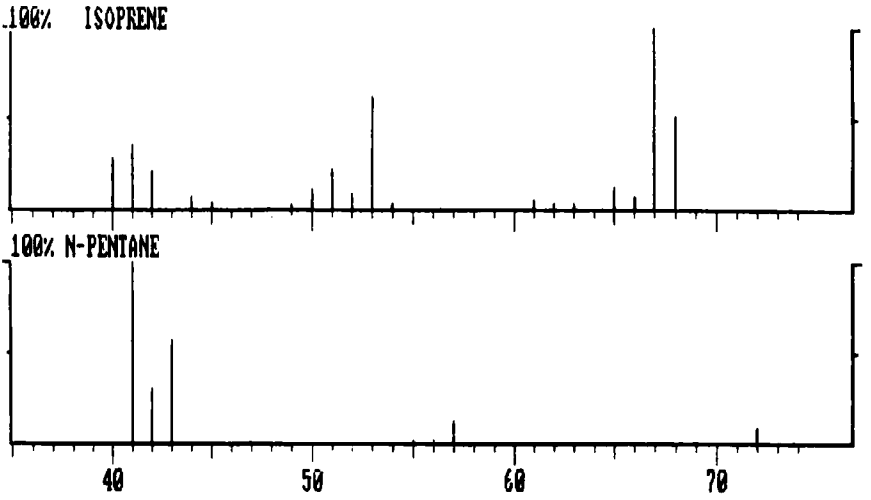

FIGURE 1 Mass spectra of isoprene and pentane.

sensitive gas chromatographic method did not detect exhalation of pentane from isolated ventilated lungs under massive peroxidative stress; Hotz[12] found that pentane levels in breath of healthy subjects were not significantly different from that in ambient air; Gelmont[29] reported that in rats pentane exhalation was not due to membrane lipid peroxidation but depended on the linoleate content of the recent diet.

CONCLUSION

Free radical formation and lipid peroxidation are mechanisms involved in many clinical situations. Evaluation of lipid peroxidation by the determination of volatile alkanes in breath has been used for several years. However, although breath samples are easy to obtain, no clinical tests are used routinely for pentane measurement. At least two analytical problems may explain this situation: the difficulty of testing human subjects in absolutely volatile-free hydrocarbon air, and the necessity of using a very specific method (mass spectrometry) to avoid possible interferences between pentane and other volatile compounds. Taking these precautions, we did not find any difference in pentane levels in breath and indoor air, so a question remains: do healthy humans expire pentane at detectable levels? Further studies are also necessary to elucidate if pentane is present or not in pathological states.

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