Correspondence

Origins of breath isoprene

A recent communication by Phillips et al. described detection of volatile organic compounds (VOCs) in human breath to discover whether these agents were produced endogenously or were inhaled from the atmosphere. Their conclusion concerning the origin of breath isoprene took us by surprise because we too have been working on the analysis and identification of this volatile hydrocarbon in expired air. Phillips et al. measured and reported what they called the "alveolar gradient" defined by them as the difference between the exhaled and the inhaled amount of the various VOCs. The alveolar gradient for isoprene was strongly negative indicating that the concentration of this hydrocarbon was considerably higher in the ambient air breathed than in the expired alveolar air, implying that isoprene has an environmental origin and is therefore not a product of metabolism. This conclusion conflicts with many other published reports identifying isoprene as the most prominent biogenic hydrocarbon expelled in the breath of humans and animals, and also emitted by plants. The isoprene in human breath is thought to be derived from the mevalonate pathway of lipid (cholesterol) metabolism. Phillips et al. also reported a negative alveolar gradient for pentane, suggesting that this hydrocarbon also originates from the environment as opposed to the metabolism. Many previous reports, however, have identified pentane in human breath as a metabolic product of lipid peroxidation.

How can the unusual results reported by Phillips et al. be explained? It should be emphasized that the actual concentration of the VOCs present in alveolar air and ambient air were not reported in absolute amounts. Any concentrations of volatile organic compounds as well as other VOCs cannot be compared with values reported in the literature. Instead, Phillips et al. determined the relative concentration of VOCs from the difference in the chromatographic response after analysing 10 litres of alveolar air and the same volume of ambient room air. Hopefully, the concentration of VOCs per litre of inhaled and exhaled air will be published shortly.

We have developed a highly sensitive and specific method for the analysis of isoprene in human breath which involves thermal desorption gas chromatography and diode array ultraviolet detection. Using this method, the concentration of isoprene in human breath ranged from 1.6 to 10.3 nmol/l (median 3.4 nmol/l) in 16 healthy volunteers. More importantly, the concentrations of isoprene in ambient room air were negligible and below the limits of detection of the method, approximately 0.02 nmol/l for a 100 ml sample. We obtained further support for a metabolic origin of isoprene by analysing air entering and leaving a respirator during treatment of a patient in intensive care. The alveolar gradient for isoprene was strongly positive, indicating that isoprene was derived from the patient as a product of metabolism. Even after sampling and analysing 5 litres of air entering the respirator, isoprene was not detectable.

Although Phillips et al. used a mass spectrometer with an ion-trap detector for analysis of VOCs, as pointed out by DeMaster and Nagasawa, the mass spectra of low molecular weight hydrocarbons are not easy to interpret because they often lack a prominent molecular ion. Whether Phillips et al. identified the VOCs by running authentic standard sub-samples or by searching a product of library mass the mass spectra or organic compounds is not clear. Because of the wide variation in GC-MS operating conditions in different laboratories, matching library spectra with the fragmentation pattern of an unknown substance is not a foolproof way to make an unequivocal identification.

We suggest that the extreme negative alveolar gradient attributed to isoprene as reported by Phillips et al. was caused by some closely related VOC, presumably an atmospheric pollutant in the room where the experiments were performed. An unusually large number of plants or other vegetation in the room where alveolar and ambient air samples were collected should be considered as one likely source of isoprene. It is widely known that isoprene is emitted into the atmosphere in relatively large quantities by plants. By letting volunteer subjects inhale compressed air from a cylinder or room air after removal of VOCs by passage through various filters will help to solve the mystery of whether isoprene is a product of metabolism or an atmospheric pollutant in the laboratory where Dr Phillips and his colleagues work.

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Dr Phillips and Greenberg comment: We thank Dr Jones and his colleagues for their pertinent comments about breath isoprene; they have prompted us to re-examine our data. Essentially, they raise two questions: did we really see isoprene in the breath? And if we did, how can one explain its negative mean alveolar gradient?

We compared the mass spectrum of the peak identified as isoprene in a typical breath chromatogram to the mass spectrum of isoprene in the computer library (fig 1). The agreement between the two confirmed the identity of the breath VOC as isoprene (2-methyl-1,3-butanediene).

We also examined the frequency distribution of the alveolar gradients of isoprene in our subjects (fig 2). They ranged over a broad isoprene synthetic range from a negative alveolar gradient, while others manufacture the compound in sufficient quantities to generate a positive alveolar gradient. This is consistent with our observations on pentane and carbon disulphide, both of which exhibited a spectrum of positive and negative alveolar gradients in groups of normal subjects.

These findings suggest a number of conclusions which might help guide future studies of VOCs in the breath. Firstly, there is a wide range of inter-individual (and possibly intra-individual) variation. Secondly, one needs to assay a VOC in the ambient air as well as in the alveolar breath to identify its...
sources. Thirdly, the alveolar gradient seems to provide a convenient index of the net effect of environmental exposure to the VOC and the rate at which it is created or destroyed in the body.

An advantage of this approach is that it obviates the need to supply the subject with chemically clean inspired air, as Jones et al suggest. We (and others) have found this to be a fruitless undertaking because of the technical difficulty of cleansing air of all VOCs at a picomolar level. It is also unphysiological, because real people breathe real air which is full of VOC contaminants.


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