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Increased N-pentane Excretion in Humans: A Consequence of Pulmonary Oxygen Exposure

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Lipid peroxidation by free radicals has been suggested as a mechanism of a lung injury caused by breathing higher than normal concentrations of oxygen. The appearance of hydrocarbons such as n-pentane in the expired gas of mammals has been proposed as *in vivo* evidence of lipid peroxidation. The excretion of n-pentane was studied in 15 healthy volunteers in whom excretion of exogenous n-pentane was determined over a 60- to 90-min period while breathing hydrocarbon-free gases. N-pentane elimination rates (mean ± SEM) in the expired gas at 0, 30, 60, 90, and 120 min were 10.2 ± 1.5, 1.6 ± 0.2, 1.2 ± 0.9, 1.3 ± 0.4, and 1.3 ± 0.3 (pmol · kg^{-1} · min^{-1}), respectively. Using a specially assembled circuit, a 2-h oxygen exposure study was performed on six healthy volunteers, in whom basal n-pentane excretion varied ten-fold among individuals, from 0.25 to 2.25 pmol · kg^{-1} · min^{-1}. After breathing 100% oxygen, n-pentane excretion was augmented 62–420% within 30 to 120 min. The authors conclude that lipid peroxidation may occur in humans within 30 min of breathing 100% oxygen. (Key words: Lung: oxygen toxicity. Oxygen: toxicity. Toxicity: free radicals; oxygen.)

SUPPLEMENTAL OXYGEN constitutes fundamental therapy for respiratory failure. However, pulmonary oxygen toxicity caused by exposure to a prolonged and high concentration of oxygen may result in deterioration of pulmonary function and ultimately death. Despite the discovery of pulmonary oxygen toxicity in the nineteenth century,[1] its pathogenesis, diagnosis, and treatment are as yet uncertain.

Recently, lipid peroxidation by free radicals has been suggested as a lung injury mechanism in oxygen breathing.[2,3] It has been proposed that the appearance of ethane and n-pentane in the expired gas of mammals is direct *in vivo* evidence of this mechanism[4]; n-pentane excretion has been observed in rats during ozone exposure, vitamin E deficiency,[5] and aging.[6] The first application of alkanes to detect lipid peroxidation in humans was made by Dillard *et al.*[7] in 1978. Surprisingly, they observed no increase in pulmonary n-pentane elimination during ozone breathing in humans, but saw large increases with muscular exercise. Stimulated by these unexpected findings, we restudied basal n-pentane excretion in humans using digital computer simulations[8] and measurements in healthy volunteers. We also studied the effects of oxygen breathing and compared metabolic production of n-pentane during air breathing with n-pentane excretion during short-term oxygen breathing.

Methods and Materials

The studies were performed on 21 healthy human volunteers (age range, 22 to 51 yr) with their informed consent. The protocols of the studies were approved by the Human Studies Committee of Massachusetts General Hospital, Boston, Massachusetts. Subjects were divided into two groups: group 1 (4 males and 11 females) and group 2 (1 male and 5 females).

Group 1. Subjects inspired a hydrocarbon-free gas mixture of 20% oxygen and 80% helium (Heliox®) for 2 h from a specially assembled breathing circuit. The inspiratory gas reservoir used was a valveless 120 l Tissot® spirometer filled intermittently with hydrocarbon-free Heliox® from compressed gas cylinders. A corrugated stainless steel tube 95 cm long and 25 mm in diameter was connected to the gas mixture *via* the inlet of a Rudolph® valve. On inspiration, the volunteer breathed the gas mixture through a 20 cm long, 25 mm diameter Silastic® tube connecting the valve to a tight-fitting mask with an air-inflatable rim. Expired gas passed through the outlet of the Rudolph® valve and into a second corrugated steel tube connected to a Maxi PEEP® valve (Boehringer Lab, Inc., Wynnewood, PA) set at 3 cmH_2O. Sampling ports for inspired and expired gas were placed in the corrugated steel tubes. Expired gas samples of 200 to 1600 ml were drawn at 0, 30, 60, 90, and 120 min after Heliox® breathing for n-pentane analysis.

Group 2. Subjects inspired Heliox® for 2 h, then hydrocarbon-free 100% oxygen for 2 h, and Heliox® for the final hour. Expired gas samples of 200 to 1600 ml were drawn every 30 min for n-pentane analysis.

The n-pentane was analyzed by gas chromatography. The hydrocarbons in large volumes of inspired and expired gas samples were trapped and then fractionally distilled onto a temperature-programmed gas chromatograph to detect trace amounts of n-pentane using a flame ionization detector (fig. 1). N-pentane elimination was calculated as the product of respiratory minute volume and the difference between expired and inspired n-pentane concentration. Nitrogen, oxygen, and carbon dioxide

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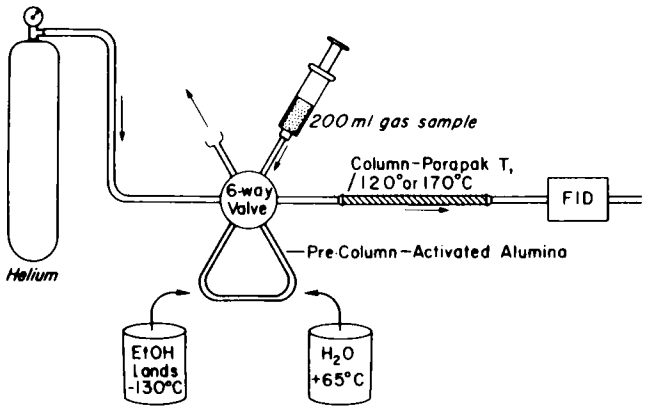

FIG. 1. Assay of n-pentane in respiratory gas. N-pentane in the sample (200 to 1600 ml) is condensed in a U-shaped precolumn, which is cooled by submersion in frozen ethanol. After low-boiling-point compounds are eliminated at a column temperature of 170° C, the precolumn is heated for analysis of n-pentane on a Porapak T® column at 120° C.

were also determined in all samples by gas chromatography.

The following procedure was used to detect contamination of the samples by environmental air. Nitrogen washout from the body was determined while the subject was breathing Heliox®. Nitrogen concentrations in the expired samples were plotted against time to obtain the nitrogen washout curve. Contamination of gas samples by environmental air was considered to have occurred when the value of nitrogen was significantly higher in the subject breathing Heliox® than that of nitrogen washout curve. The Wilcoxon Signed-rank test (nonparametric test) was used to analyse the data for statistical significance.[9]

Results

Group 1. Pertinent data are summarized in table 1 and figure 2. Minute ventilation ($\dot{V}_E$) at 0 min was higher than the standard value, perhaps due to stress of the volunteers. All breathed room air for 5 min by mask before breathing Heliox®. Heliox® contained less than 0.01 nl/l of n-pentane. N-pentane elimination rate (mean ± SEM) in the

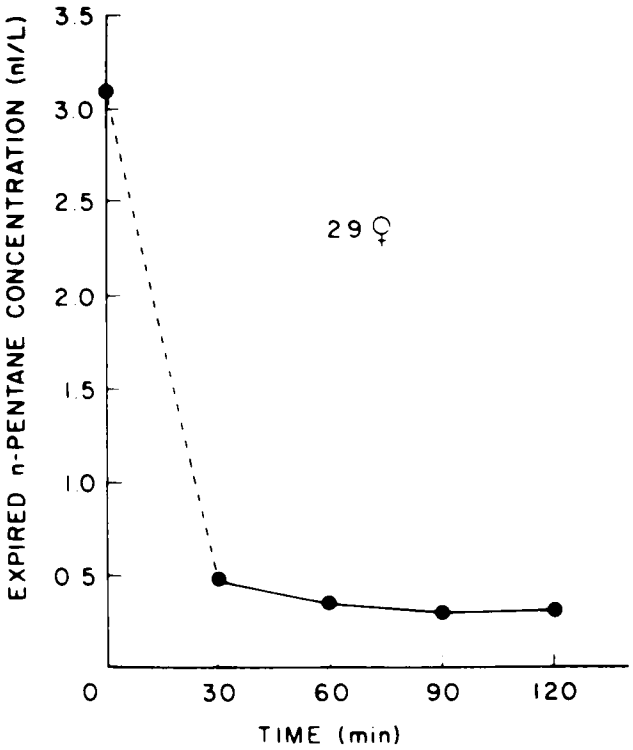

FIG. 2. A representative n-pentane washout curve of a volunteer. The value of expired n-pentane at the beginning (0) and at 60 and 90 min were 3.1 nl/l, and 0.35 and 0.35 nl/l, respectively. Note expired n-pentane decreased from the initial value and became a constant value at 60 to 90 min.

expired gas ($\dot{m}p$) at 0, 30, 60, 90, and 120 min were 10.2 ± 1.5, 1.6 ± 0.2, 1.2 ± 0.9, 1.3 ± 0.4, and 1.3 ± 0.3, ($pmol \cdot kg^{-1} \cdot min^{-1}$), respectively. Figure 2 shows a representative n-pentane washout curve. The initial level of the inspired n-pentane concentration was 3.1 nl/l and decreased over 60 to 90 min to a constant value of 0.35 nl/l.

Group 2. The basal n-pentane excretion among subjects varied ten-fold from 0.25 to 2.25 $pmol \cdot kg^{-1} \cdot min^{-1}$. All volunteers increased their n-pentane excretion during oxygen breathing by 62 to 420% (table 2). Significant responses occurred from 30 to 120 min from the onset of oxygen breathing ($P < 0.02$). Figure 3 illustrates a

TABLE 1. Basal N-pentane Excretion in Healthy Volunteers at Rest (mean ± SEM)

	Time (min)				
	0	30	60	90	120
$\dot{V}_E$ (l/min)	8.57 ± 0.46	7.09 ± 0.26	6.63 ± 0.22	6.98 ± 0.25	6.88 ± 0.26
E_p (nl/l)	1.77 ± 0.27	0.32 ± 0.04	0.26 ± 0.17	0.27 ± 0.07	0.27 ± 0.05
$\dot{m}p$ ($pmol \cdot kg^{-1} \cdot min^{-1}$)	10.2 ± 1.5	1.6 ± 0.2	1.2 ± 0.9	1.3 ± 0.4	1.3 ± 0.3

$\dot{V}_E$ = minute ventilation; E_p = n-pentane concentration in the expired gas; $\dot{m}p$ = n-pentane elimination rate in the expired gas.
pmol = 10^{-12} mole.
Heliox® contained less than 0.01 nl/l of n-pentane.

TABLE 2. N-pentane Excretion Rate before and after 100% Oxygen Breathing (pmol · kg^{-1} · min^{-1})

Basal N-pentane Excretion during Heliox	Augmented N-pentane Excretion during Oxygen
0.50	2.25
0.75	1.50
0.25	1.05
0.40	1.55
0.80	1.30
2.25	3.85

$P < 0.2$ by Wilcoxon Signed-rank Test

representative response during oxygen breathing. N-pentane excretion increased during oxygen breathing and decreased during Heliox® breathing.

Discussion

Oxygen-generated free radicals are highly reactive[10,11] and may result in lipid peroxidation,[2] and ultimately in oxygen toxicity.[12,13] Workers have succeeded, at present, in measuring not the quantity of lipid peroxidation *per se* or the free radical reaction *per se,* but the quantity of lipid peroxide. One of the methods used in these attempts is to quantitate hydrocarbons in the expired gas to detect amounts of lipid peroxide produced by free radicals.[4,14] It is generally accepted that alkanes such as n-pentane in human breath originate from the decomposition of lipid peroxides and reflect free-radical reactions in the body if those from environmental sources are excluded. Ethane and n-pentane are ubiquitous air pollutants and are taken up through the lungs, skin, and intestinal mucosa. Because physiologic stress may mobilize tissue stores of these alkanes, the increased pulmonary excretion of n-pentane during muscular exercise may not represent lipid peroxidation but merely the passive washout of previously equilibrated exogenous, environmental n-pentane from muscle caused by increased blood flow. A study on ozone exposure and muscular exercise[7] reported waiting 5 min for washout before adding ozone and observing no change. However, the further washout of n-pentane in the previously reported study might offset the production by lipid peroxidation. All previous studies in humans and animals confused the excretion of exogenous and endogenous n-pentane. Our primary experimental maneuver was to wash out the environmental, exogenous n-pentane by hydrocarbon-free gas as demonstrated in Group 1. The n-pentane washout curve was transformed to a logarithmic scale and dissected into three compartments (fig. 4). The initial drop was interpreted as the washout of nonmetabolic, environmental n-pentane from the functional residual capacity. The second compartment represented the slow washout of nonmetabolic n-pentane from viscera, muscle, and fat. The final plateau value was taken as the basal production of metabolic n-pentane. To reach a point where n-pentane excretion represents basal metabolic production by lipid peroxidation, one must wait 60 to 90 min.

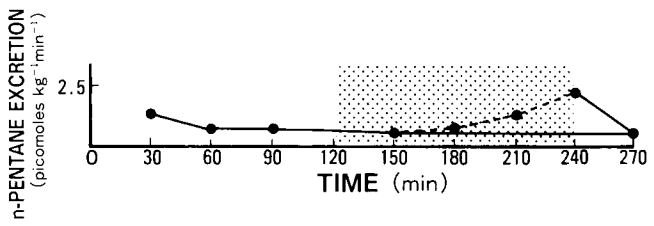

FIG. 3. A representative response in n-pentane excretion in a volunteer. N-pentane excretion increased during oxygen breathing (*shaded area* and *dashed line*) and decreased to a base line after Heliox® breathing (*solid line*).

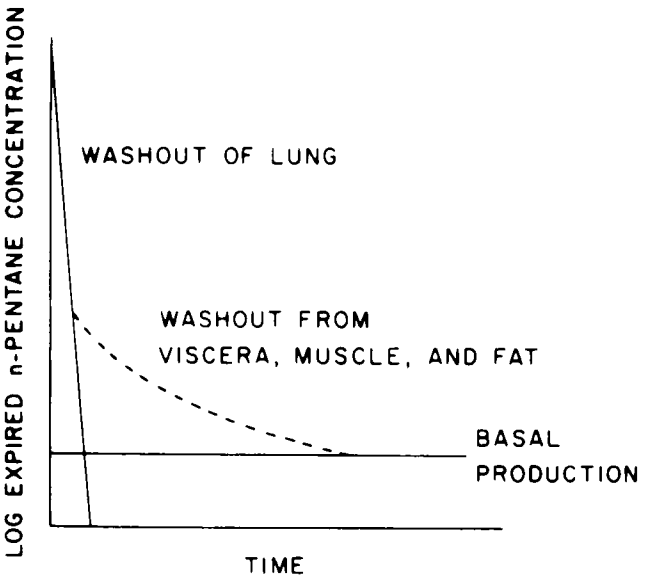

FIG. 4. Theoretical curve of n-pentane washout: Factors influencing alkane excretion.

The reliability of data obtained in this study depends on the breathing circuit. The circuit should be leak-tight and composed of as much nonabsorbable, nonleachable material as possible. In addition, if samples are contaminated with environmental air, they should be detected as such and be separated to avoid gross error. A new breathing circuit composed primarily of metal and water was constructed to meet these requirements. In our preliminary study, however, a breathing circuit composed of plastic materials was used; the plastic materials absorbed and leached out hydrocarbons, resulting in a substantial dissociation between the *in vivo* phenomena and levels of n-pentane detected in breath, and making difficult the collection of accurate information on free radical reactions in the body.

Findings of the present study demonstrated that the augmented excretion of endogenous n-pentane during 100% oxygen breathing occurred as early as 30 min and that the phenomenon was reversible, as evidenced by the decrease in n-pentane after Heliox®. It is suggested that lipid peroxidation may occur in man within 30 min of breathing 100% oxygen. However, the point at which lipid peroxidation overwhelms repair mechanisms and produces irreversible oxygen toxicity injury remains unknown.

Today, many patients receive high levels of inspired oxygen in order to correct severe arterial hypoxemia during lung disease. Under these conditions, oxygen toxicity may develop. Until a dose–response relationship between inspired oxygen tension and n-pentane excretion is established and the lung is shown to be the site of peroxidation, the amount of oxygen and antioxidant therapy given to patients receiving respiratory care cannot be rationally titrated.

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