

### A micro apparatus for absorption of ethylene and its use in determination of ethylene in exhaled gases from human subjects

It has been known for many years that ethylene is evolved by ripening fruit and certain microorganisms<sup>1</sup>. Recently KAKANOV<sup>2</sup> detected ethylene in air passed through respiratory chambers which contained rats or swine ascarids, but no precautions were taken to maintain aseptic conditions in the chambers. We wish to report the hitherto unsuspected presence of ethylene in exhaled breath of human subjects. An apparatus for the quantitative collection of ethylene in total amounts as low as a fraction of a  $\mu\text{l}$  is described.

The ethylene-absorption apparatus is shown in Fig. 1. It contained 4.5 ml of mercuric perchlorate (0.25 M in 2.0 M  $\text{HClO}_4$ ), an absorber specific for olefins<sup>3</sup>, with 5  $\mu\text{l}$  of *n*-butanol as a foaming agent. A continuous stream of purified<sup>4</sup> air was passed

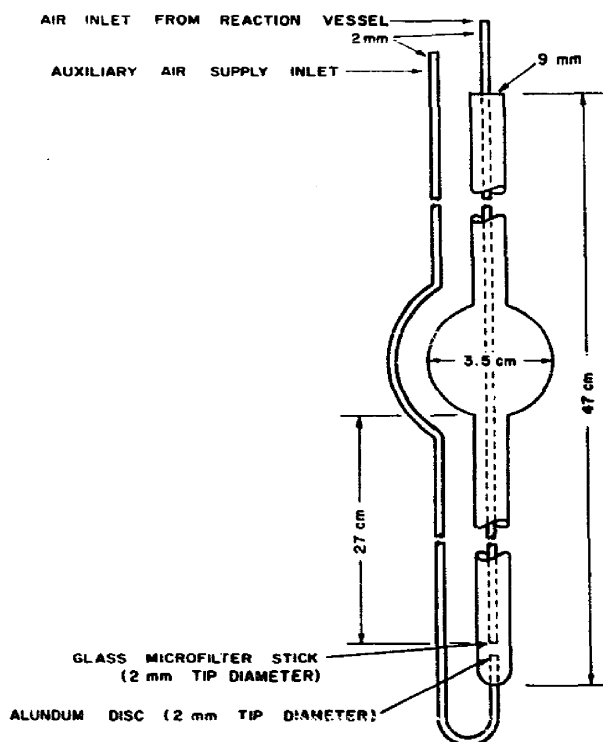


Fig. 1. Absorption tube for collection of ethylene.

through a reaction vessel containing ethylene and into the absorption apparatus. For testing the system's efficiency in ethylene recovery, the low biological rate of ethylene evolution was simulated by regeneration of the gas from its mercury complex by gradual addition of 4 M LiCl. Ethylene was determined by a gas-chromatographic procedure described earlier<sup>5</sup>.

Results of recovery experiments with different concentrations of ethylene, various flow rates and natures of absorption column are summarized in Table I. Glass filter sticks (Microchemical Specialties Co., Cat. No. 7310, medium or fine porosity) gave a much coarser dispersion than alundum disc filters. It is evident from Table I that

the ethylene-collecting efficiency depended on the fineness of dispersion of the foam, the length of the foam column, and the gas-flow rate.

Where it is important to prevent the build-up of pressure inside a reaction vessel, the absorption tube shown in Fig. 1 may be used (Expts. 9-13). When the alundum disc is connected to an auxiliary, purified air supply flowing at the rate of 20 ml/min,

TABLE I  
EFFECT OF FLOW RATES AND NATURE OF ABSORPTION COLUMN ON  
EFFICIENCY OF ETHYLENE COLLECTION

Expt.	Filter stick	Flow rate (ml/min)	Absorption column:		Ethylene added ( $\mu$ l)	Ethylene recovery (%)
			Nature of dispersion	Length (cm)		
1	Glass	35	Coarse	10	0.07	0
2	Glass	30	Coarse	10	0.24	0
3	Glass	26	Coarse	5	0.24	30
4	Glass	26	Coarse	5	0.07	34
5-8	Alundum	26	Fine	11-15	0.02-0.24	100-107
9-13	Glass/Alundum*	5/20*	Fine	10-13	0.02-0.24	100

\* Oblique bars signify the combination of glass and alundum filters and the respective flow rates through them.

it will maintain a 10-13-cm column of finely foaming mercuric perchlorate solution. The reaction vessel is then connected to the absorption tube through a glass filter stick and air is passed through at the rate of 5 ml/min. This apparatus has proved useful for studies of ethylene production by sub-cellular fractions from fruit<sup>5</sup>.

TABLE II  
ETHYLENE CONTENT OF ROOM AIR AND EXHALATIONS OF ADULT HUMAN SUBJECTS

Source	Ethylene content (parts/10 <sup>6</sup> )
Room air	6.7
Subject 1 (female, fasted)	—
Subject 1 (female, fed)	18.0
Subject 2 (male, fasted)	24.3
Subject 3 (male, fasted)	25.0
Subject 3 (male, fed)	—

With this high-efficiency absorption tube we investigated the ethylene content of the exhaled gases from human subjects. Expired gases from adult male or female subjects, all non-smokers, fasted 10 h or unfasted, were collected in a 100-l Douglas bag and then pumped at the rate of 25 ml/min into mercuric perchlorate solution through the alundum filter disc. Control samples with room air or outside air were collected in the Douglas bag and analyzed similarly. Each determination for ethylene was made from 40-50 l of gas. Confirmation of the identity of the gas as ethylene was obtained mass spectrometrically for representative samples. The results of analysis of exhaled gases respired indoors are summarized in Table III. While the

limited number of experiments does not permit evaluation of the effects of sex, age, physical condition etc., it is clear that the exhaled gases of human subjects are richer in ethylene than the room air inhaled by them. It is known that with fruits, generation of ethylene is an autocatalytic process<sup>1</sup> and the presence of ethylene in room air may act as a stimulant to ethylene production by humans.

For a more quantitative relationship between the ethylene contents of exhaled gases and inhaled air several experiments were conducted with one individual (male, non-smoker). The exhaled gases were collected 4 h after the evening meal, outside the building in the open air, away from possible contamination from such sources such as automobile exhaust. Inhaled air contained ethylene at an average concentration of  $3.46 \pm 0.02$  (standard error)  $\cdot 10^{-3}$  parts/million (6 determinations) whereas exhaled air contained ethylene at  $6.79 \pm 0.13$  (6 determinations). The difference between these means was statistically highly significant. These results indicate that the ethylene content of the exhaled gas of the normal adult subject is nearly double that of the air inhaled by him.

The production of ethylene is probably not solely attributable to the gut flora, since we have obtained ethylene from subcellular fractions from rat liver and rat intestinal mucosa (unpublished data from G. RAM CHANDRA AND M. SPENCER).

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### Competitive inhibition of corticoid synthesis by estrogens

When estrogens are incubated with rat adrenal tissue they inhibit the synthesis of corticoids<sup>1</sup>. Administration of high levels of estrogens *in vivo* also inhibits production of corticoids<sup>2</sup>. Since glucose-6-phosphate dehydrogenase (EC 1.1.1.49) is inhibited by estrogens, it seemed possible that the regulation of the rate of reduction of NADP is a mechanism in the regulation of adrenal function<sup>3</sup>. Estrogen has an inhibitory effect on other NADP-specific dehydrogenases and at lower levels estrogen stimulates NAD-specific lactate dehydrogenase (EC 1.1.1.27)<sup>4</sup>. However, it has been demonstrated that the generation of NADPH<sub>2</sub> by glucose-6-phosphate dehydrogenase is of special significance in corticoid synthesis<sup>5</sup>. The competitive inhibition by estrogens of the rate of reduction of added NADP in adrenal homogenates has now been correlated with the competitive inhibition to corticoid synthesis. In studies of this kind an application of kinetic principles is essential.

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