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Gas Chromatography of Organic Volatiles in Human Breath and Saliva

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Samples of human breath and head space vapours over saliva have been analyzed in a gas chromatograph equipped with a flame ionization detector. A simple direct sampling method without any enrichment procedure has been used. At the highest sensitivity of the chromatograph about ten different peaks could be registered. Individual variations and the influence of food, alcohol and smoking have been demonstrated. The effect of a preparation for oral hygiene has also been shown. A typical chromatographic pattern for breath and saliva vapours has been constructed. The analytical technique presented could be applied to other problems in medicine and biochemistry.

The rapid evolution of gas chromatography over the last decade has made possible the separation and detection of trace amounts of gaseous components, *e.g.* in air. In the field of odontology, this technique offered a new and promising way for the study of gases and organic volatiles in breath and saliva. Using an ionization-type detector, MacKay, Lang and Berdick [1] were able to record chromatograms of human breath. The major peaks in these they assumed to represent acetone and ethanol. In 1961, Weber and Rhoades at USAF Aerospace Medical Center, Texas,[2] reported that they had found acetone, methanol and ethanol in human saliva. Finally, Eriksen and Kulkarni [3] in 1963, stated that the breath of all persons they tested contained methanol. In all these investigations, the samples were enriched prior to analysis by various procedures, such as the use of pre-columns or condensation at liquid-air temperatures.

In the present work, it has been possible to record reproducible chromatograms of the organic volatiles in breath and over saliva using a simple, direct sampling method. The samples were taken with a heated, all-glass syringe which prior to analysis was thoroughly cleaned in steam, and rinsed in purified nitrogen. The syringe was then checked for purity using the purified nitrogen as a test substance. Very low bleeding of the column permitted the use of the highest sensitivity of the chromatograph with an unsplit stream of carrier gas.

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EXPERIMENTAL

Apparatus. The gas chromatograph used in the investigation was equipped with a commercial flame ionization detector, Perkin Elmer 116 E, whereas the column system and the thermostat were constructed in this laboratory, *cf.* Widmark.[4] The column, sealed directly onto the T-shaped injection port, was made of Pyrex glass-tubing 1.75 m, 3.5 mm i.d. in the form of a spiral of five turns. Connections to detector and gas supply consisted of short stainless tubings, 2.3 mm o.d. fitted into Nicols silicone stoppers. The injection port, separately heated, was closed with a Nicols "blindhole" stopper easily punctured at injection. The carrier gas, helium, was led through a trap containing activated molecular sieve, Linde 5 Å.

The electrometer was manufactured by Perkin Elmer, type 116 E. The recorders used were Leeds & Northrup Speedomax 1 mV and Siemens Kleinkompensograph 2.5 mV. Syringe: 10 ml, all glass, (Chance). Sampling bottles: 50 ml, glass with rubber serum caps.

Reagents. Ascoxal (Astra), specially prepared without flavouring.

Operating conditions: Injection port 120°C. Column thermostat 82°C. Detector thermostat 130°C. Helium flow 50 ml/min. Hydrogen flow 35 ml/min. Air flow 350 ml/min.

Stationary phase. Polyethyleneglycol (M = 1500) 20 % on Chromosorb W, bled to give baseline stability when employing the highest sensitivity of the electrometer.

Students and laboratory personnel of both sexes have acted as subjects.

Cleaning procedures. Prior to sampling, the syringe and sampling bottles were boiled in distilled water, dried and stored in closed containers. Before use, the syringe was heated in an electrical oven (80°C) and rinsed by filling with pure nitrogen several times. A blank obtained by injecting 10 ml pure nitrogen, demonstrated by Fig. 1 was acceptable; otherwise, the syringe was recleaned.

Sampling and injection procedures

1. Expired air (alveolar air). After breathing fresh air by an open window the subject slowly exhaled through the mouth, while an alveolar air sample (10 ml) was sucked from the innermost part of the oral cavity *via* an injection needle (7 cm) into the heated (80°C) syringe. The sample was immediately injected into the injection port of the chromatograph.

2. Mouth air. The subject closed his lips around the injection needle and filled the mouth with inhaled, fresh air. This air volume was moved around in the oral cavity for 15 seconds by the use of lips, tongue and cheeks. The mouth air sample (10 ml) was then sucked into the syringe and injected.

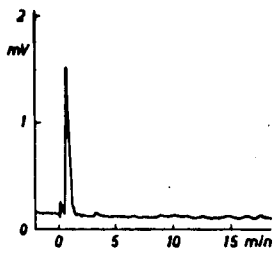

Fig. 1. Chromatogram obtained by injecting 10 ml purified nitrogen.

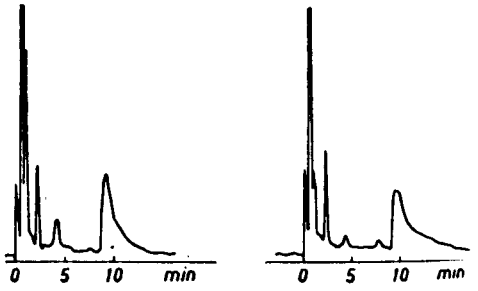

Fig. 2. Chromatograms of expired air from two subjects. 10 ml samples were taken in the morning and the subjects did not eat, drink, smoke or brush their teeth before sampling.

3. Saliva vapour. 3–5 ml unstimulated saliva was collected in the glass sampling bottle, which was closed with a rubber serum cap. After heating in an electrical oven (60°C) for 5 min, a 10 ml head space vapour sample was sucked into the syringe *via* an injection needle through the rubber serum cap. To adjust the pressure, air was allowed to enter through a short second injection needle. The system was checked for purity prior to sampling.

RESULTS

The chromatograms obtained in this investigation show that about ten different organic volatiles can be detected in samples of human breath and saliva vapour by the method described. Clear differences in the occurrence and quantities of these compounds in samples from different individuals can be seen. To illustrate this, two actual chromatograms of expired air from two subjects are shown in Fig. 2. The samples have been taken in the morning and the subjects did not eat, drink, smoke or brush their teeth before sampling. Fig. 3 shows chromatograms of saliva vapours from two subjects. The individual variations can also be seen here.

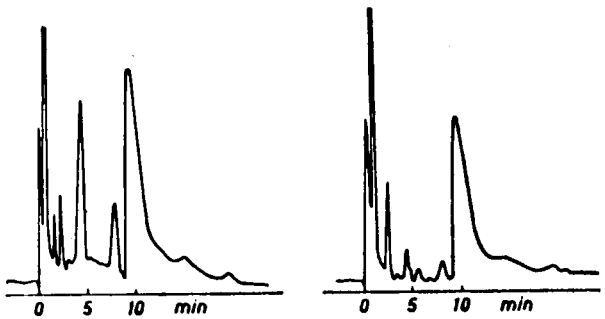

Fig. 3. Head space vapours over fresh saliva from two subjects. Sample volume 10 ml, temperature 60°C.

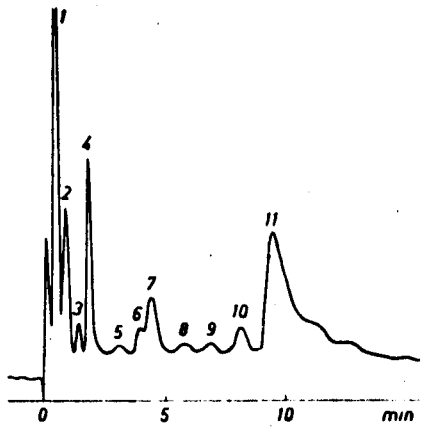

Fig. 4. Chromatogram constructed to show the typical arrangement of peaks from volatiles in breath and saliva vapours. 1, air. 3, acetaldehyde. 4, acetone. 6, methanol. 7, ethanol. 11, water.

In spite of these variations, a certain pattern can be recognized in all chromatograms of volatiles in breath and saliva vapours. Such a chromatogram has been constructed in Fig. 4 showing the typical arrangement of peaks. The quantitative composition given resembles that of the most common types. The peaks have been numbered and a tentative identification of four peaks, air and water not included, is indicated. These peaks are assumed to represent acetaldehyde, acetone, methanol and ethanol. Evidence for identification of these components was limited to a comparison of retention times of previously identified compounds. Therefore it has to be stressed that this identification is only tentative.

Various foodstuffs have influence on the composition of breath. In Fig. 5 the first chromatogram shows mouth air after the subject has eaten an apple.

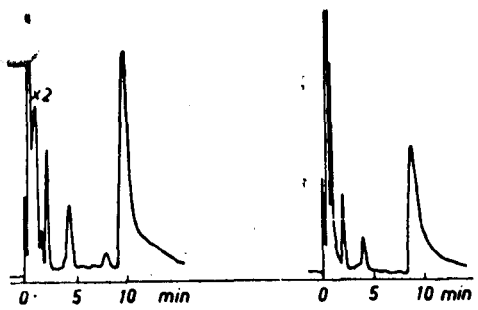

Fig. 5. The first chromatogram is from mouth air of a subject who has eaten an apple. The second shows the same after the use of a preparation for oral hygiene (Ascoxal).

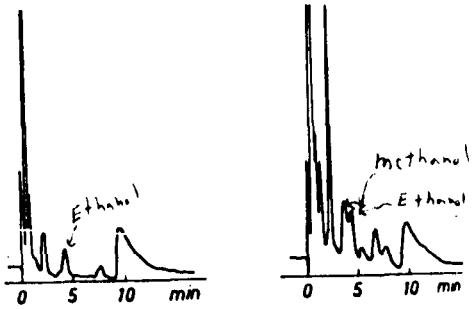

Fig. 6. Chromatograms of expired air before and after the subject's smoking a cigarette.

The second shows the same subject's mouth air after the use of a preparation for oral hygiene with oxidative and microbicidal properties (Ascoxal, Astra).

Fig. 6 shows chromatograms of expired air before and after smoking a cigarette. The second sample was taken one minute after the last inhalation, to prevent influence of residual smoke.

Table 1 shows the rise and fall of the ethanolic peak in chromatograms of expired air from a subject after the intake of 5 cl 45 % ethyl alcohol. None or little variation can be seen in the other peaks.

DISCUSSION

Gas chromatography has been used in a few studies of the volatiles in breath and saliva. Due to the small amounts of the compounds in question, enrichment techniques of various types have been tried, such as condensation at liquid air temperatures and the use of enrichment columns. More sensitive detectors, larger sampling volumes and columns free from hampering bleeding has made possible the method described in this investigation, *i.e.* direct sampling with a heated all-glass syringe. Simplicity and rapidity are the main advantages of the method. The detection of subtle variations in the volatile content of breath and saliva in different individuals is possible and may prove useful in the study of factors that are related to general and oral pathology. Thus the fact that acetone is always present in human breath and is easily detected and measured by gas chromatography ought to be a valuable contribution to the diagnosis and study of diabetes.

Table 1. Peak heights before and after a subject's intake of 5 cl 45 % ethyl alcohol.

Peak number	Time in minutes							
	Before	After						
	10	10	25	40	65	85	110	170
	Peak heights in mm							
2	20	21	21	20	18	23	23	12
4	10	16	23	23	18	16	19	19
7	6	196	252	224	208	200	120	18

Ethanol has also been detected in all chromatograms of human breath. This fact conforms with the findings of MacKay *et al.*[4] On the other hand, Lester,[5] who concluded that with the use of gas chromatography ethanol could be detected in breath samples at concentrations equivalent to 0.5 mg per liter blood, found ethanol in the breath of 15 persons of 25 tested. The question of the origin of this endogenous ethanol is an interesting one which is still unanswered.

Methanol has in this investigation only infrequently been detected in samples of human breath, a fact contradictory to the results of Eriksen and Kulkarni.[3] They found methanol and ethanol in breath of all persons tested and mostly more methanol than ethanol. Whether the presence of methanol might be explained from a bacteriological standpoint or as the result of some metabolic process is not clear.

Although chromatograms of breath and saliva volatiles can now easily be obtained and may provide very useful information, the interpretation of such chromatograms is a very difficult undertaking. Due to the low concentrations of the components present even the absolute identification of the various peaks presents serious problems. Nevertheless, at the present stage of this method, no other analytical technique equals gas chromatography as an approach to the study of volatiles in breath and saliva. Finally, it should be emphasized that the technique described here ought to be applicable to similar problems in other fields of medicine and biology.

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