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Endogenous Production of Methanol after the Consumption of Fruit

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After the consumption of fruit, the concentration of methanol in the human body increases by as much as an order of magnitude. This is due to the degradation of natural pectin (which is esterified with methyl alcohol) in the human colon. In vivo tests performed by means of proton-transfer-reaction mass spectrometry show that consumed pectin in either a pure form (10 to 15 g) or a natural form (in 1 kg of apples) induces a significant increase of methanol in the breath (and by inference in the blood) of humans. The amount generated from pectin (0.4 to 1.4 g) is approximately equivalent to the total daily endogenous production (measured to be 0.3 to 0.6 g/day) or that obtained from 0.3 liters of 80-proof brandy (calculated to be 0.5 g). This dietary pectin may contribute to the development of nonalcoholic cirrhosis of the liver.

Key Words: Mass Spectrometry, Trace Analysis, Methanol in Breath, Endogenous Methanol, Breath Tests.

THIS WORK reports on the increase of methanol concentration in the blood after consumption of fruit. Because methanol is assumed to be a precursor for liver cirrhosis, this raises the question of whether the continuous consumption of fruit can contribute to the development of nonalcoholic cirrhosis.

The blood and total body water of human beings contain endogenous concentrations of ethanol and methanol of typically 0.2 to 0.8 mg/liter and 0.5 to 2 mg/liter, respectively.[1,2] (These values correspond to 0.05 to 0.20 ppm of ethanol and 0.15 to 0.6 ppm of methanol, respectively, in the breath.) These concentrations are of endogenous origin, which is still incompletely understood. Partly, they may arise as fermentation products in the gut[3]; however, ethanol has been identified in the blood and tissue from germ-free rats, indicating endogenous formation independent of the microflora in the gut.[4] Also, methanol is a trace product of mammalian intermediary metabolism.[5,6] A typical human body produces up to 30 g of ethanol/day[7] and 0.3 to 0.6 methanol/day. This amount is obtained from an endogenous methanol production of (0.3 ± 0.1) mg/liter $\cdot$ hr^{-1} reported by Gilg et al.,[8] which was also confirmed in the present investigation.

Although ethanol concentrations keep within the aforementioned limits unless exogenous ethanol is introduced to the body, the level of methanol increases as much as an order of magnitude a few hours after consumption of various kinds of fruit.[9,10] Concentrations as high as 3 ppm in the human breath, corresponding to ~10 mg/liter in the blood, have been observed in test persons eating 0.75 kg of peaches and/or apples. Corresponding blood methanol concentrations were reported recently by Gilg et al.[8] and Grüner et al.[11] (Already in 1941, Werch and Ivy[12] were able to show that up to 90% of the pectin added to a mixed diet, in the case of normal dogs, disappears and does not show up in the feces. Similar results were obtained with normal humans. In contrast, recovery of pectin from ileostomy material, obtained from dogs ranged from 84 to 89%, and human ileostomy material yielded a pectin recovery ranging from 94 to 97%. These findings showed, that most, if not all of the pectin decomposed during the passage in the alimentary tract is decomposed in the colon.) These enhanced methanol levels originate from the degradation of pectin in the human colon. Pectin is a source of methanol: natural pectin consists of joined galacturonic acid units, some of which are esterified with methyl alcohol—ranging from 30% in grapes typically to 75% in apples.[13] Pectin is degraded in the colon, and there is evidence to show that this is done by bacteria. Siragusa et al.[14] have demonstrated in vitro that pectin is degraded by fecal bacteria, and methanol is released by this degradation. By 72 hr, fecal flora cultures and bacteria *E. carotovora* cleaved 30 to 90% of all possible methoxyl groups in pectin-glucose substrates. In vivo experiments performed by Grüner et al.[11] have shown that ingestion of 13.3 g of pectin by test persons done three times a day over a period of 2 days, followed by ingestion of 0.5 g/kg of ethanol (free of any methanol), resulted in an increase of the serum methanol concentration up to a maximum of 50 mg/kg. As will be seen, our results are consistent with this. In this present work, we used a newly developed mass spectrometric method to perform quantitative in vivo tests on the release of methanol in the human body after the consumption of fruit and pure pectin.

METHODS AND MATERIALS

Ethanol and methanol concentrations were measured in the human breath using proton-transfer-reaction mass spectrometry (PTR-MS) in a selected ion flow drift tube (SIFDT).[9] PTR-MS has successfully measured the concentration of methanol, ethanol, and acetone in human breath,[9,10] as well as that of benzene and acetonitrile in the breath of smokers and nonsmokers.[15] Most recently, we have detected strongly enhanced levels

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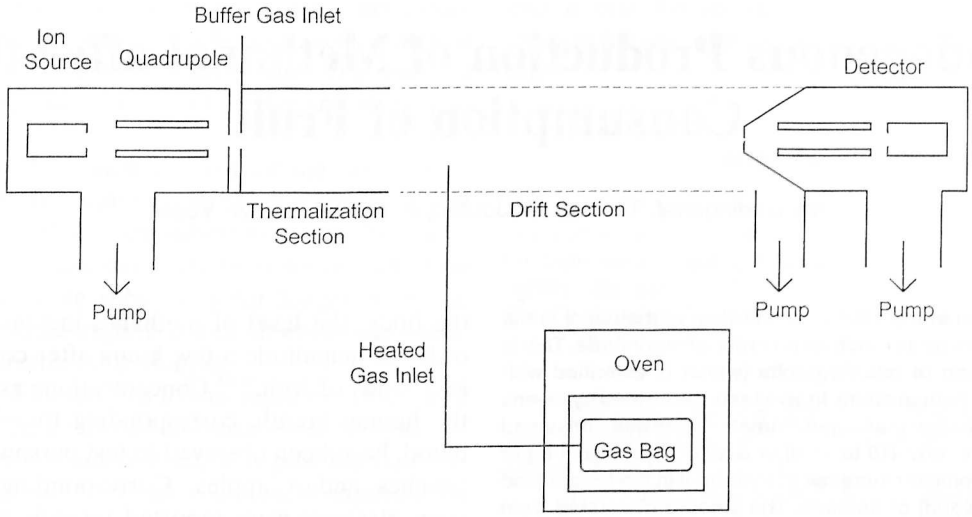

Fig. 1. Schematic representation of the SIFDT.

of allylmethylsulfide, dimethyl sulfide, diallyl sulfide, diallyl trisulfide, and other volatile organic compounds in the human breath of persons after consumption of garlic.[16]

Figure 1 shows a schematic representation of the SIFDT apparatus. Briefly, H_3O^+ ions produced in a high-pressure ion source are mass selected and injected into a drift system operating with helium buffer gas of ~0.2 torr at 300 K.[8] Herein, the ions are thermalized in collisions with the helium atoms (i.e., the ions reach thermal equilibrium with the buffer gas being at room temperature, and travel downstream under the combined influence of the buffer gas flow and the electric field applied in the drift section). A flow of typically 5 STP $cm^3\ sec^{-1}$ of the breath gas to be analyzed is added to the helium buffer gas through a heated inlet and H_3O^+ ions, colliding with trace gas components from the breath sample, undergo fast proton transfer reactions with rate constants of $\sim 2 \times 10^{-9}$ $cm^3\ sec^{-1}$ on the way from the gas inlet to a downstream sampling orifice. Both primary ions H_3O^+ and protonated products are then detected in a downstream quadrupole mass spectrometer system and from the count rates of the primary and product ions, the concentrations of the trace constituents are calculated, as shown by Lagg et al.[9] and Hansel et al.[17], using the relation

$$[RH^+] = [H_3O^+]_o(1 - e^{-k[R]t}) \cong [H_3O^+]_o[R]k{\cdot}t, \qquad (1)$$

where $[H_3O^+]_o$ is the density of H_3O^+ ions without the presence of reactant neutrals in the buffer gas, and k is the reaction rate constant for the proton transfer reaction.

$$H_3O^+ + R \xrightarrow{k} RH^+ + H_2O. \qquad (2)$$

Reaction rate constants are reported for many proton transfer processes in the literature.[17] t is the average time or "reaction time" the ions spend in the reaction region.[9,17] In the case of [R] denoting small densities of trace constituents, then $[RH^+] \ll [H_3O^+] \approx [H_3O^+]_o$ = constant. The ion detection system measures count rates $i(H_3O^+)$ and $i(RH^+)$ that are proportional to the respective densities of these ions. A high ion count rate $i(RH^+)$ per unit density [R] in the gas to be analyzed is therefore indicative of a high sensitivity of the system. This obviously can be achieved by keeping the density $[H_3O^+]$ high. For the present investigation, wherein the densities of the constituents to be measured are in the ppm range, primary ion count rates of 20,000 to 50,000 cps are quite sufficient. The typical uncertainties for the measured concentrations are ±20%. We want to mention, however, that for the investigation of trace components in breath and air being at the ppb level, a new more sensitive PTR-MS system has already been developed and is described in detail by Hansel et al.[17] Checks for the present investigations also have been made using this new PTR-MS system, showing excellent agreement with data obtained by the SIFDT apparatus. The high sensitivity of the new PTR-MS system is demonstrated by a typical mass scan shown in Fig. 2, as obtained from a breath sample of a healthy person.

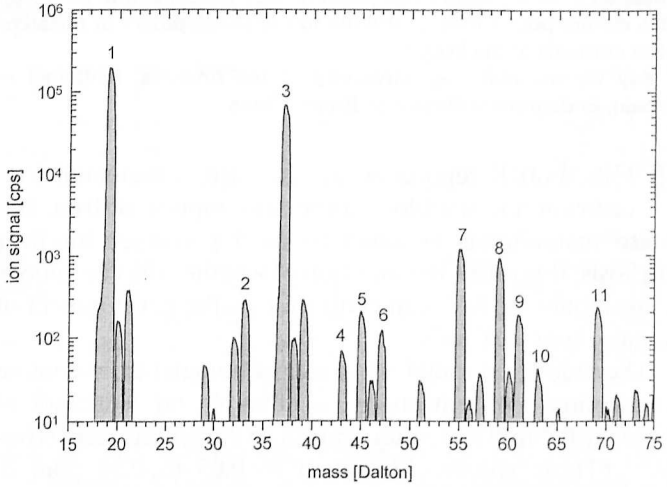

Fig. 2. Mass scan obtained from the breath sample of a healthy person. Mass peaks 1 through 11 correspond to protonated ions RH^+, with R being: 1, H_2O; 2, methanol; 3, $(H_2O)_2$; 4, propanol fragment; 5, acetaldehyde; 6, ethanol; 7, $(H_2O)_3$; 8, acetone; 9, acetic acid; 10, dimethyl sulfide; and 11, isoprene.

Table 1. Typical Densities of Various Trace Components in the Breath of a Healthy Person, as Obtained from the Mass Scan Shown in Fig. 2

No.	Compound	Mass	Density (ppb)
1	H_3O^+, primary ion	19	—
2	Methanol	33	395
3	$H_3O^+ \cdot H_2O$	37	—
4	Propanol	43	126
5	Acetaldehyde	45	184
6	Ethanol	47	222
7	$H_3O^+ \cdot (H_2O)_2$	55	—
8	Acetone	59	533
9	Acetic acid	61	117
10	Dimethyl sulfide	63	36
11	Isoprene	69	616

Table 1 shows the concentrations of several components obtained from the mass scan in Fig. 2. Mass 37 represents the cluster ion $H_3O^+ \cdot H_2O$ and the peak at mass 32 represents O_2^+ – impurities. More detailed

investigations on the concentrations and identification of breath components shown in Table 1 are reported by Taucher et al.[15] and Hansel et al.[17]

An equilibrium is established between ethanol in the blood and ethanol in the breath,[4]

$$\text{BEC[g/liter]} = \text{BrEC[g/2,100 liter]}, \quad (3)$$

where [BrEC] is the mole fraction of ethanol in the breath in ppm and [BEC] is the concentration of ethanol in the blood (g/liter). We estimate the total amount of ethanol in the body, M(E), in grams from

$$[\text{BEC}] = \frac{\text{M(E)}}{\text{W} \times 0.70}, \quad (4)$$

where W is the weight of the human body in kilograms. The value 0.70 represents the average volume of distribution for ethanol in the body for males and uses the unit liters/kilogram. A similar equilibrium is established for methanol according to the relation[4]:

$$\text{BMC[g/liter]} = \text{BrMC[g/2,709 liter]}. \quad (5)$$

Thus, the concentrations of methanol and ethanol in the breath are quantitative estimates of the total amount of methanol and ethanol present in the human body.

We are interested in measuring quantitatively the release of methanol in the human body after the consumption of fruit. The baseline concentration of methanol in the body reflects a balance between endogenous production and metabolic loss. It has been well established that the metabolic loss of methanol is completely stopped, when the human body contains an elevated concentration of ethanol.[2,18–22] This allows the measurement of the endogenous baseline methanol production to be typically 0.28 mg/liter · hr^{-2} or ~0.3 g/day for a 70 kg man. This same technique may be used to measure methanol production when fruit is consumed, because the methanol production is even greater. These measurements have been made on different kinds of fruit and on pure pectin.

All investigations performed during this work were started between 9 and 10 AM. All test persons were male and in a fasted state. The measurements were done in three steps.

In the first experiment, two subjects (27 and 31 years old; 90 and 79 kg, respectively) were given 75 g each of a mixture of pure ethanol and distilled water (40%/60%) at the beginning of the experiment and again 2½ hr later to maintain an ethanol concentration in the breath above 40 ppm and thus in the blood well in excess of 150 mg/liter throughout the 5½ hr of duration of the experiment. The methanol concentration in the breath of the test persons (as well as of ethanol) was measured by PTR-MS in intervals of ~30 min.

In the second experiment, performed 1 day later, four subjects (including two from the first experiment) of 22, 27, 31, and 52 years of age were kept under the same conditions (with respect to the blood ethanol concentration) as the subjects in the first experiment, now for ~12 hr (taking ~60 to 70 g of the above water-ethanol mixture every 2 hr), but were also fed 10 g of pectin (one exception was 15 g) with a degree of methylization of 75% at the beginning of the experiment. Methanol (as well as ethanol) concentration in the breath was measured again every half an hour over the total test period of ~12 hr.

In the third experiment, performed 3 days after the second experiment, the identical four test persons performed the same experiment with the *only* exception that instead of pure pectin they now ate ~1 kg apples each at the beginning of the experiment. Apples typically contain ~1% pectin,[21] with a degree of methylization of ~75%. Thus, a typical amount of 1.3 g of methanol is contained in the pectin of the 1 kg apples consumed. Again the methanol (and ethanol) concentrations in the breath of the test persons was measured throughout the duration of the experiment for ~10 hr.

RESULTS

The results of the first experiment are presented in Fig. 3, showing the breath methanol concentration for the two test persons involved as dependent on time. The initial concentrations of 0.30 and 0.35 ppm, respectively, are typical basis methanol concentrations, in agreement with earlier previously described observations[1,2,8]; but, due to the intake of ethanol by the test persons right at the beginning of the experiment, the now enhanced ethanol concentration in the body inhibited methanol metabolism, thus causing the observed increase of the breath methanol concentrations as a function of time. The graph yields a value for the increase of the methanol concentration of 0.15 ppm/hr, which corresponds to ~0.6 g/day and is in fair agreement with the findings of Gilg et al.[2,8]

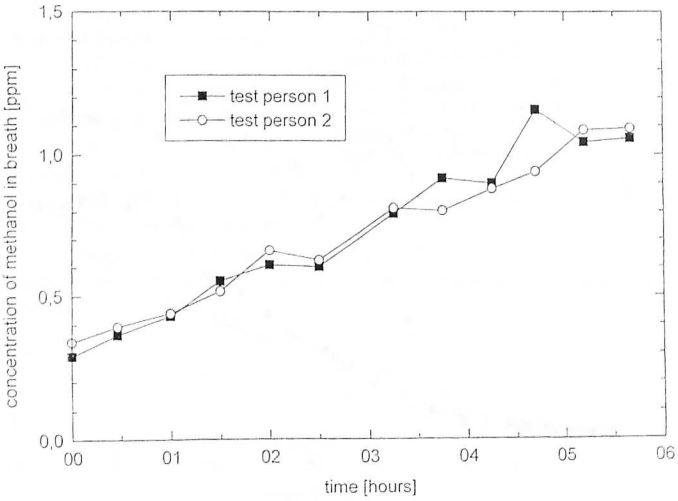

Fig. 3. Increase of the concentration of methanol in the breath of two test persons after consumption of pure ethanol at time 0 and 2½ hr (maintaining an ethanol concentration in the blood in excess of 150 mg/liter throughout the measurements), which inhibits methanol metabolism.

In the second experiment, wherein pectin was consumed in addition to the ethanol-water mixture, during the first 3 to 4 hr the increase of methanol (Fig. 4) occurred at a rate typical for the normal endogenous methanol production as observed in the first experiment. After that, however, a considerably stronger increase of the breath methanol concentration was observed, due to the degradation of the pectin in the lower intestines. From data in Fig. 4, we estimate the concentration of methanol produced from the pectin as the difference between the total methanol concentration (full lines with symbols) and that produced endogenously (dashed line). This difference reaches a steady value of 4 to 8 ppm after 7 to 10 hr. From this, we calculate an amount of 0.4 to 1.4 g of methanol present in the body of the test persons solely due to the release of methanol from pectin. This result shows that ~30 to 70% of the total amount of methanol bound in the pectin that was consumed was actually released in the body.

In the third experiment, wherein apples were consumed instead of pectin, the respective amount of methanol released as calculated from the measured breath methanol concentration shown in Fig. 5 is ~32 to 56% of the total amount of methanol bound in the pectin of the consumed apples. Additionai measurements performed with six test

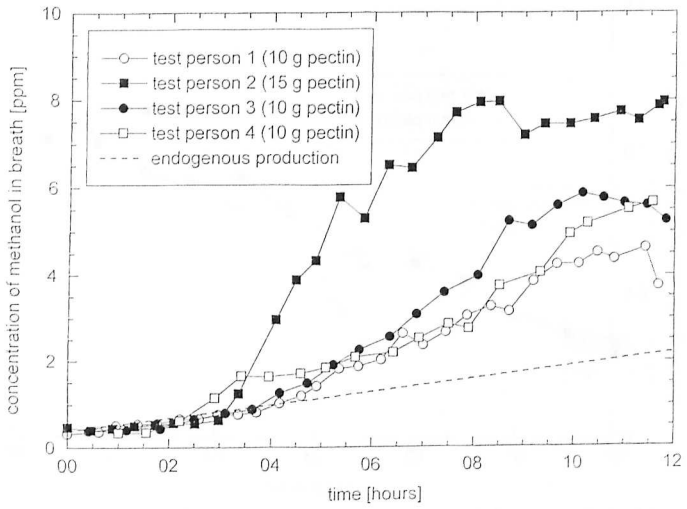

Fig. 4. Increase of the concentration of methanol in the breath of four test persons after consumption of pectin (having a degree of methylization of 75%) at time 0. Over the whole time period of the experiment, ethanol concentration in the blood was kept in excess of 150 mg/liter.

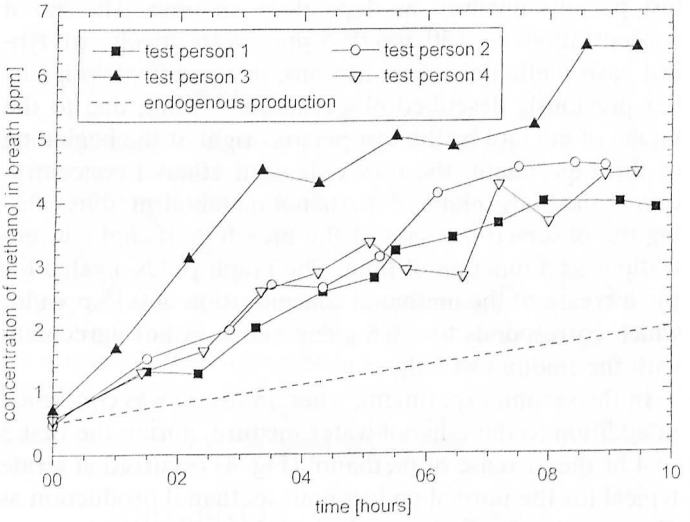

Fig. 5. Increase of the concentration of methanol in the breath of four test persons (same as in Fig. 2) after consumption of 1 kg apples each at time 0. Over the whole time period of the experiment, ethanol concentration in the blood was kept in excess of 120 mg/liter.

persons (data not shown in Fig. 5) yielded fractions of methanol release between 25 and 52%. Total statistics, including all 10 persons tested, show a release of 40% ± 9% of methanol contained in the consumed apples. Herein, the main uncertainty lies in the content of ~1% of pectin in apples,[23] which is subject to a change of ±50%. Therefore, we may assume that between 20% and 100% of all the methanol contained in the pectin of the consumed apples are finally resorbed in the body.

DISCUSSION

The results herein show that, after consumption of fruit, the methanol bound in the fruit pectin is released quantitatively and transferred to the blood. Thus, after consumption of 1 kg of apples a total of typically 0.5 g of methanol is released in the human body. Therefore, the daily consumption of a few apples or oranges increases the endogenous production of methanol over the normal one (being 0.3 to 0.6 g/day) by about a factor of 2.

To acquire the same quantity of methanol, a person has to drink 0.3 liters of brandy (40% ethanol) containing 0.5% of methanol (compared with ethanol), which would qualify as significantly methanol-contaminated liquor.

These results on methanol release after consumption of fruit are of particular interest with respect to the nonalcoholic fatty liver disease, the pathophysiology of which is not understood.[24] To our knowledge, no statistical data exist on the correlation between nonalcoholic fatty liver disease and the habits of the patients with respect to consumption of fruit—data that would be highly desirable in view of the present findings.

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