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ORIGINAL ARTICLE

# Ethyl glucuronide concentrations in beard hair after a single alcohol dose: evidence for incorporation in hair root

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**Abstract**
*Background* Despite the growing importance of ethyl glucuronide (EtG) in hair for detection of chronic excessive alcohol consumption, the mechanism of incorporation is not yet clear. Deposition from sweat is believed to be the main route. In order to get more information, EtG was determined in daily shaved beard hair after single higher alcohol doses.
*Methods* Three volunteers drank within 5.5 h 153, 165 and 200 g ethanol followed by abstinence. Daily shaved beard hair was analysed for EtG using a validated liquid chromatography–tandem mass spectrometry method with a limit of quantification of 2 pg/mg.
*Results* For all three volunteers, small concentrations of EtG were already detected 9 h after end of drinking. The concentrations increased to maxima of 182, 242 and 74 pg/mg on days 2 to 4 and then gradually decreased to limit of quantification on days 8 to 10.
*Discussion* The time course of EtG is discussed based on literature data about anatomic dimensions of the hair root, physiology of hair growth, kinetics of EtG formation and elimination in blood, and in comparison to literature results about drugs in beard hair. It follows that for beard hair the predominant portion of EtG is incorporated in the upper part of the hair root between suprabulbar region and isthmus leading to a positive zone of about 3 mm (8–9 days) after a single drinking event. Deposition from sweat which is only possible into the residual hair stubble after shaving and in the infundibulum down to the sebaceous gland mouth was found to be of minor importance but could play a greater role in long hair.
*Conclusion* It is concluded that EtG in hair fulfils the prerequisites for time-resolved interpretation of segmental concentrations and that a single excessive drinking can be well detected in sufficiently short hair segments. However, in the routinely investigated 3-cm proximal scalp hair segment and using the cutoff of 7 pg/mg, a negative result can be expected with high probability because of dilution by negative hair.

**Keywords** Alcohol marker · Beard hair · Ethyl glucuronide · Hair analysis · Incorporation mechanism

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## Introduction

Ethyl glucuronide (EtG) in hair has gained increasing practical importance as a marker of chronic excessive alcohol consumption [1–6]. Comparison of this minor metabolite of ethanol with traditional alcohol markers such as the activity of gamma-glutamyltransferase or the concentration of carbohydrate-deficient transferrin showed clearly higher sensitivity and specificity [7–9]. Despite these advantages and growing application, the incorporation mechanism of EtG into hair is not yet fully known. Because of its anionic state at physiological pH and of its hydrophilicity, a low incorporation rate from blood into the hair root was estimated in comparison to basic and lipophilic drugs. Sebum as a

possible incorporation way of lipophilic substances can be excluded for the hydrophilic EtG. But, since EtG is excreted with sweat [10], deposition from sweat was assumed to be the essential way of incorporation. On the other hand, it was concluded by Kharbouche et al. [11] that the bloodstream is likely to display a major role in the incorporation of EtG into hair since EtG was detected in the hair of ethanol-fed rats which have no sweat glands in their fur.

Analysis of beard hair collected by daily shaving after a single dose was shown to be a suitable way to investigate the incorporation mechanism [12–18]. In case of incorporation from blood in the hair root, the substance should be detected with a delay of several days when the incorporation zone of the steadily growing hair had appeared on the skin surface whereas in case of deposition from sweat the detection should be possible already some hours after administration. Therefore, in the present study, EtG was determined in daily shaved hair of three volunteers after a single higher alcohol dose followed by abstinence.

## Materials and methods

### Chemicals and reference substances

Ethyl glucuronide and ethyl glucuronide-d5 were purchased from Promochem GmbH (Wesel, Germany). Acetonitrile (for gradient HPLC quality) was obtained from Fisher Scientific, Schwerte, Germany. All other reagents were obtained in analytical grade purity from Merck (Darmstadt, Germany).

### Drinking experiment and collection of beard hair samples

After abstinence of more than 2 weeks, three volunteers (authors of this paper and their colleagues) drank a higher dose between 153 and 200 g ethanol within 5.5 h in the night of day 0 to day 1. Details are given in Table 1. After the end of drinking, a blood sample was taken for determination of blood alcohol concentration (BAC). Beard hair was collected by shaving with an electric razor each morning. Collection details are also given in Table 1. No remaining stubbles were felt after shaving. Caused by the working principle of the electrical razor (lift and cut technique), individual hairs are lifted and can be cut even below the skin surface. Not all hairs are uniformly trimmed. This source of uncertainty is neglected in the present paper and it is assumed that the hair was cut on skin surface level.

The hair clippings were removed from the razor head and stored at room temperature in aluminium foil until analysis. After collection, the razor head and interior were carefully cleaned. During the study, the face was cleansed only with water and no cosmetics (crèmes, face lotions, etc.) were applied.

The total amount of each daily beard shaving (between 9.1 and 43.6 mg) was washed in a 1.5-mL Eppendorf vial subsequently with 1 mL dichloromethane for 15 min and 1 mL methanol for about 30 s. Water (0.5 mL) and EtG-d5 (10 ng) in 50 μL water/acetonitrile (90:10, *v*/*v*) were added to the washed hair and ultrasonicated for 30 min. After that, the mixture was incubated overnight at room temperature (23 °C). The extract was filtered into a glass vial using a syringe filter (4 mm, RC membrane 0.45 μm, Phenomenex, Aschaffenburg, Germany).

### Determination of EtG by LC-MS/MS

The validated liquid chromatography–electrospray ionisation–tandem mass spectrometry (LC-ESI-MS/MS) method for determination of EtG was described in detail in a previous paper [2] and was now improved by using a new instrument with higher sensitivity. The measurement was performed with a Triplequad LC-MS/MS instrument Agilent 6490/1290 (Agilent Technologies, Waldbronn, Germany) equipped with a Thermo Hypercarb Column (100× 2.1 mm, 5 μm particle size, ThermoFisher Scientific, Dreieich, Germany). Chromatography was achieved under isocratic conditions with acetonitrile/0.1 % formic acid in water (8:92, *v*/*v*) as the mobile phase, a flow rate of 0.3 mL/min and a run time of 6 min. The injection volume was 20 μL and the retention time of EtG was 3.6 min.

After optimization, the following MS/MS conditions were used: ESI in negative mode, capillary voltage of 4,300 V, nozzle voltage 500 V, drying gas 120 °C/14 L/min, sheath gas 400 °C/10 L/min and nebulizer pressure 60 psi. For multiple reaction monitoring, the following transitions (collision energy) were used with a dwell time of 80 ms each: EtG 221.2>75.1 (10 V), 221.2>85.1 (13 V); EtG-d5 226.2>75.1 (14 V) and 226.2>85.1 (13 V). The transitions leading to *m*/*z*=

**Table 1** Drinking data and collection protocol of beard hair for investigation of EtG

| Volunteer no. | Age, years | Drinking time, days 0–1 | Ethanol dose, g | BAC, g/kg on day 01:00 h | Time of beard hair sampling | | | Hair amount per day, mg |
|---|---|---|---|---|---|---|---|---|
| | | | | | Day 0 | Day 1 | Days 2–32 | |
| 1 | 53 | 07:30–01:00 hours | 153 | 1.25 | 10:00 | 10:00 | 06:30–07:30 | 34.7±4.7 |
| 2 | 44 | 07:30–01:00 hours | 200 | 1.30 | 10:00 | 10:00 | 06:30–07:00 | 33.6±5.5 |
| 3 | 39 | 07:30–01:00 hours | 165 | 1.50 | 11:00 | 11:00 | 06:00 | 26.2±6.3 |

75.1 were used for quantification. For a hair sample of 30 mg, the limit of detection (LOD)=0.5 pg/mg and the limit of quantification (LOQ)=2 pg/mg were estimated from the signal to noise ratios of 3 and 10 and were confirmed for real samples with concentrations in this range.

## Results

The data of the three volunteers, the drinking amounts and the blood alcohol concentrations at the end of drinking are shown in Table 1. Between 153 and 200 g ethanol were drank within a single session of 5.5 h. The alcohol concentrations in the blood at the end of the session were between 1.25 and 1.5 mg/g. The daily amounts of beard hair available for analysis were between 9.1 and 43.6 mg. The variations can be explained from incomplete collection from the razor head rather than from varying hair growth. Thorough examination of the samples showed the presence of a minor portion of epidermis scales among the hair which could not be removed. It is assumed in the further discussion that their contribution to results has no essential effect on the interpretation.

The time course of the EtG concentrations in daily shaved beard hair samples is shown in Fig. 1. Results between LOD and LOQ were included for a complete demonstration of the time course despite their high uncertainty. For all three volunteers, EtG could be detected on day 1 (about 14 h after begin of

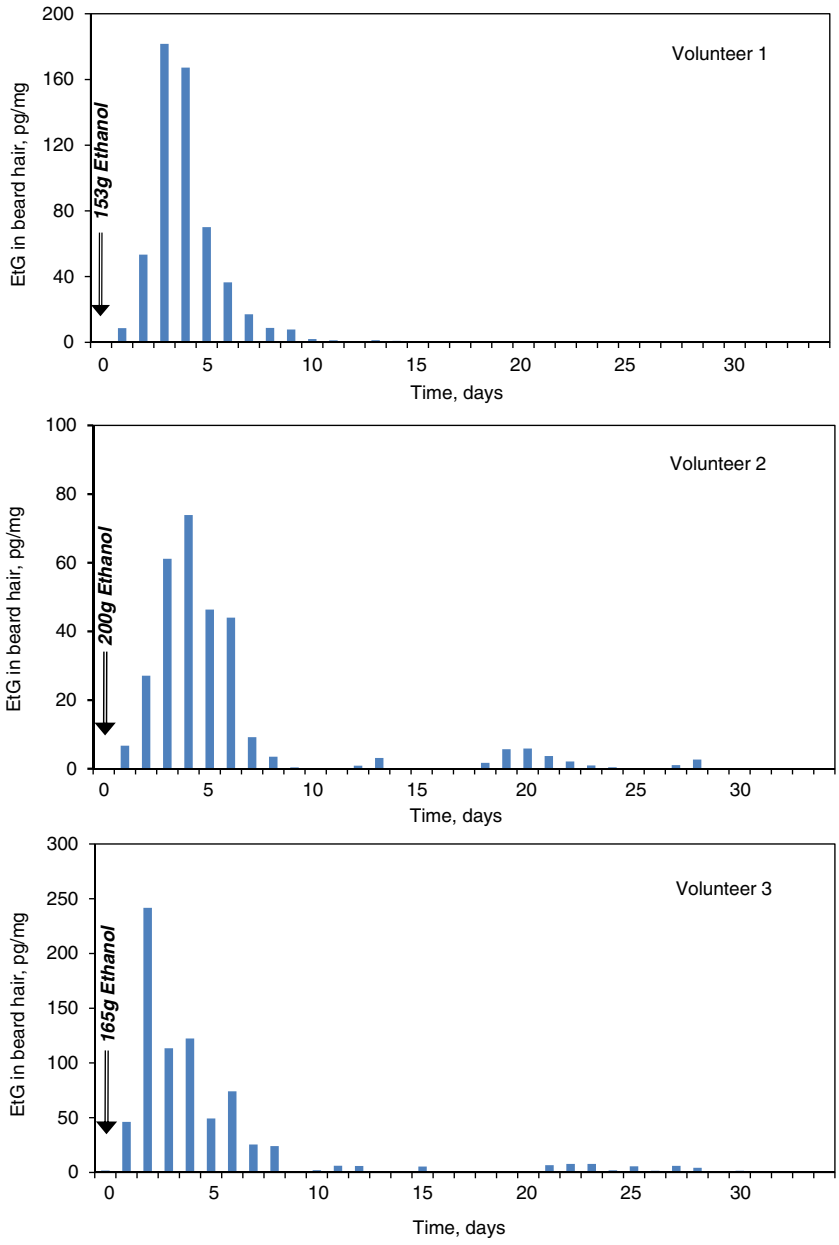


**Fig. 1** EtG concentrations in daily shaved beard hair of three volunteers after single high alcohol doses

drinking) with concentrations between 6.7 and 46 pg/mg. For volunteer 1, the highest concentrations were measured on days 3 and 4 (182 and 167 pg/mg). Then it gradually decreased to 7.8 pg/mg on day 9, and very low concentrations above LOD were still estimated on day 14. A similar time course was seen for volunteer 2 with the highest concentrations (44 to 74 pg/mg) between day 3 and day 6 and measurable concentrations above LOQ until day 8. The increase between day 18 and day 23 is caused by a bottle of beer (20 g ethanol) about 2 weeks after the drinking session which was later admitted by the volunteer. The samples from volunteer 3 provided an extremely high concentration (242 pg/mg) already on day 2 with a gradual decrease until day 8. This volunteer admitted small drinks as a reason of the low EtG values after day 10 too.

## Discussion

### Anatomy of hair root and physiology of hair growth

The knowledge about the time course of the incorporation of a substance from blood into hair is based on the anatomy of the hair root, on the physiology of hair growth and on the pharmacokinetics of the substance [19–28]. A schematic diagram of an anagen terminal hair root is shown in Fig. 2. From anatomic point of view, it can be divided along its axis from below to the skin surface into the *hair bulb* including the papilla, the *suprabulbar region* up to the arrector pili muscle, the *isthmus* between the point of attachment of the arrector pili muscle and the point of entry of the sebaceous gland duct, and the *infundibulum* above the entry of the sebaceous duct to the surrounding epidermis surface.

Along this way, the hair fibre is formed. Around the papilla on the basement membrane the matrix cells of medulla, cortex and cuticle are located. In this lowest part of the follicle, a rapid cell proliferation and differentiation occurs. The rapid mitosis forces a migration of the upper zones in the direction of the mouth of the hair root. In the next higher zones, the genes for formation of keratin are expressed, followed by keratinisation and incorporation of pigments. Further up in the suprabulbar region, hardening, disulfide bonding, resorption and dehydration occur, all cytoplasmic organelles disappear and the residues of the cells are coupled to membrane structures. At the level of the entrance of the sebum duct, the inner root sheath degenerates and gives room for the sebum in which the mature hair is bathed for 2 or 3 days until it reaches the skin surface.

The time of hair development from the matrix cells to skin surface can be calculated from the length of the follicle and the hair growth rate. In general, hair follicles vary strongly in size and literature data about their dimension are rare. The length of terminal scalp hair follicles from basement cells to skin surface was determined as 3.86± 0.61 mm by Vogt et al. [21] and between 3 and 5 mm by Pötsch [24]. Furthermore, Vogt et al. determined the position of the sebaceous gland mouth below the skin surface (length of infundibulum, 0.58±0.08 mm) and of the lower

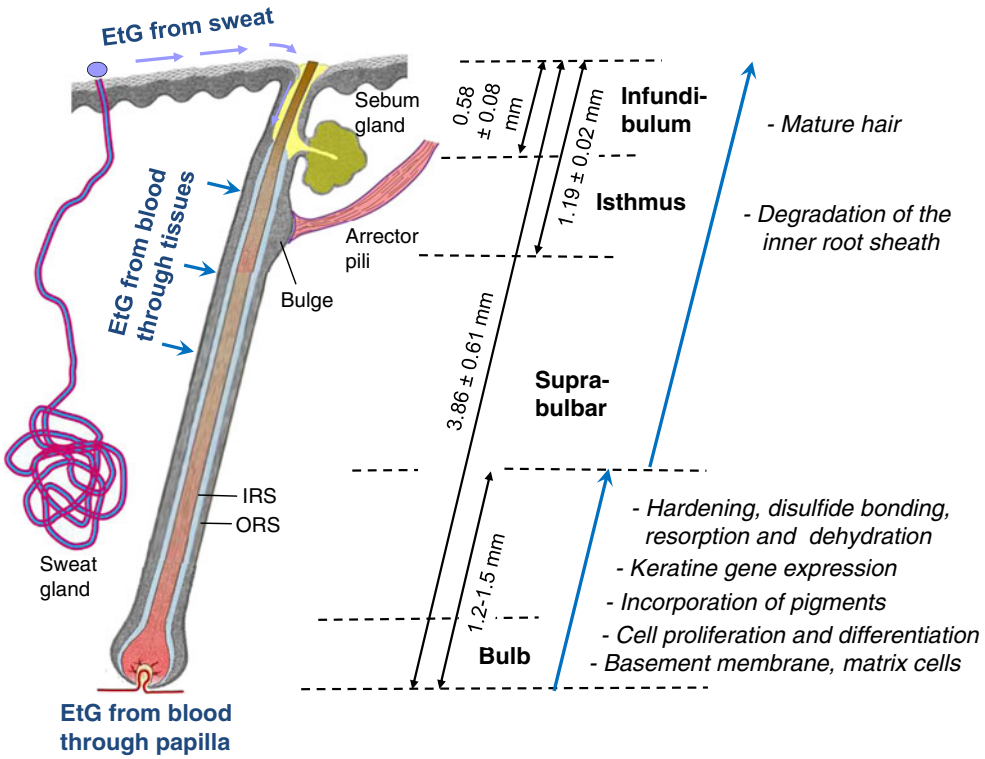


**Fig. 2** Schematic diagram of a shaved anagen terminal hair root and steps of hair formation. The given dimensions originate from Vogt et al. [21] and Pötsch [24]. According to Pötsch [25], the hair fibre is completely keratinized at a level of 1.2 to 1.5 mm above the papilla. *IRS* inner root sheath, *ORS* outer root sheath

end of the bulge region (region of attachment of the arrector pili muscle, 1.19±0,02 mm) as well as the length of the bulge region (0.24±0.05 mm) for terminal scalp hair follicles [21]. According to Pötsch [25], the hair fibre is completely keratinized at a level of 1.2 to 1.5 mm above the papilla. In the "Richards–Meharg body and head hair growth table" [29], slightly smaller hair follicle depths of 2–4 mm were described for beard hair.

The growth rate of beard hair is according to Randall and Ebling 0.25–0.29 mm/day [30] and according to the Richard–Meharg table 0.32–0.38 mm/day [29] and may also vary strongly since Nagle described an average of 0.47 mm/day for pigmented hair and of 1.12 mm/day for white hair of three individuals over a time period of 3 years [31].

## Time window of EtG in blood and in sweat

The exposure time of the hair root to EtG in blood and in sweat should have an essential effect on its distribution in hair shavings. It is a limitation of the present investigation that the time course of EtG in blood of the three volunteers was not measured and literature data must be used. According to pharmacokinetic studies by Droenner et al. [32], Høiseth et al. [33, 34] and Halter et al. [35], EtG is detectable in blood from less than 1 h after the start of drinking until up to 6 h after completed elimination of ethanol depending on dose. The maximum concentration of EtG was found to be about three orders of magnitude lower than that of ethanol [33]. Based on these data, it can be grossly estimated for the three volunteers from the drinking time of 5.5 h and the BACs at the end of drinking (1.25 to 1.50 g/kg, see Table 1) with an ethanol elimination rate of 0.15 g/kg/h that EtG should have been present in the blood for approximately 22 h from about 14 h before shaving 1 to about 8 h after shaving 1.

No data about the excretion kinetics of EtG in sweat were found in literature. Schummer et al. [10] applied sweat patches for 3 to 12 h including drinking time (73±24 g ethanol) to 14 volunteers for EtG collection and determined concentrations of 23.0±12.6 μg/L in sweat after correction to the sodium content. This is the averaged concentration over the collection time and is about two orders of magnitude below the EtG concentration in blood. The excretion time of EtG in sweat cannot be derived from the results. Therefore, the mechanism of eccrine sweat production and studies with other exogenic substances were reviewed.

The eccrine sweat glands in the scalp and beard hair regions form sweat in an active process from blood plasma. Exogenic compounds present in the blood such as alcohol [36], gamma-hydroxybutyric acid [37], opiates [16, 38], cocaine [39] or amphetamines [40, 41] are also excreted with eccrine sweat. According to the physiological mechanism of eccrine sweat production [42, 43], the excretion of these substances should occur only as long as they are present in the blood and a delayed excretion cannot be expected. Accordingly, drugs were detected in sweat patches as early as 2 h after intake and the majority was excreted within the first 24 h [38–41].

However, small amounts of methamphetamine [40] and 3,4-methylenedioxyamphetamine [41] were also detected in sweat patches up to 7 days or even longer after cessation of intake. This prolonged detection of the more lipophilic drugs is probably not caused by freshly formed sweat but by sebum and by liberation from tissue depots which cannot be separated from sweat by these patches. This cannot be assumed for the hydrophilic EtG. Therefore, the detection time of EtG in blood (about 22 h after begin of drinking in the present study, see above) can be assumed to be also the approximate duration of excretion of EtG in sweat.

## Incorporation in hair from blood

According to the prevailing concept, it is believed that substance incorporation from the blood into the hair occurs through the papilla in the lower part of the follicle up to the zone of keratinisation, hardening and dehydration of the hair matrix [25, 44] (Fig. 2). Assuming a follicle depth of 3–4 mm and a growth rate of 0.3 mm/day, the total transfer time of an incorporated substance from the matrix cells to the mouth of the root should be about 10–13 days. It could be expected from the position of the completed keratinisation (1.8 to 2.5 mm below the skin surface [25]) that the predominant amount appears in the shavings later than 6 to 8 days after drug intake.

For verification of this concept in the case of EtG, in Fig. 3 the concentrations in daily shavings are arranged in the approximate position of the corresponding hair root sections at the time of alcohol consumption for the example of volunteer 1. The results for volunteers 2 and 3 are similar. Differently from expectation, it is seen that most of the substance is durably deposited into the completed hair in the upper suprabulbar and isthmus regions and only a very small portion in the region of hair matrix formation. This does not considerably change if a higher growth rate or a shorter follicle depth is assumed. It can be explained by the fact that the hair root is exposed to EtG only as long as it is present in blood (about 22 h). During this time, it is distributed by passive diffusion through the papilla and through the root sheaths to all parts of the hair root including the upper suprabulbar and isthmus regions where the solidified hair matrix should still be swelled with water to enable diffusion. After that, EtG is eliminated back into the blood particularly from the well-perfused lower parts of the root leading to very low concentrations there, whereas it remains stored with high concentrations in the solid higher parts. The diffusion back into the blood is not prevented by binding to

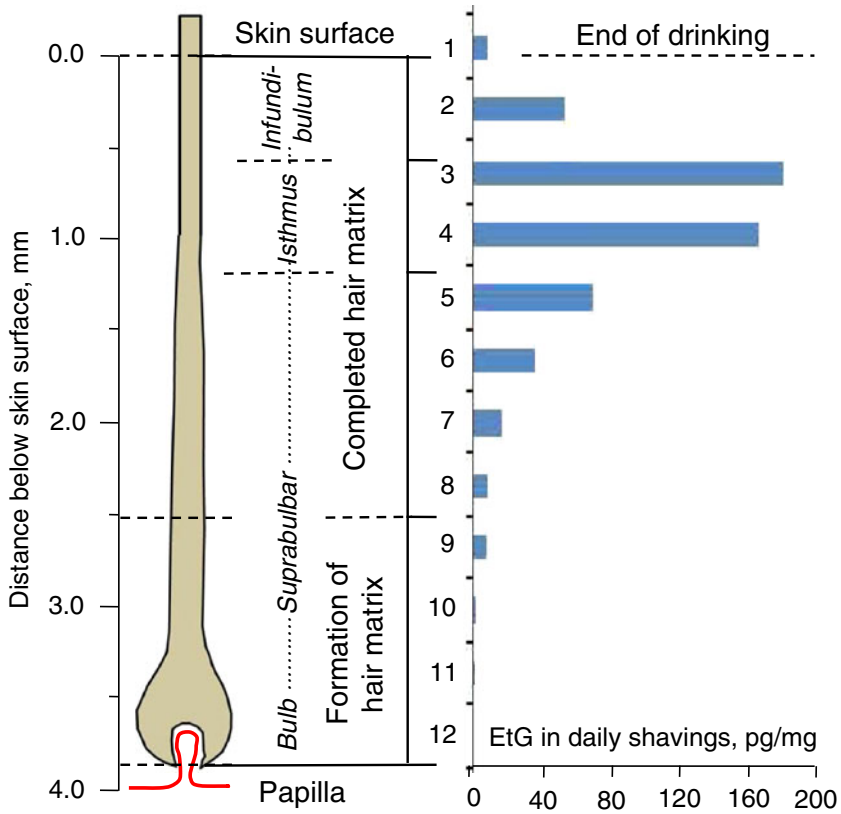


**Fig. 3** EtG concentrations in daily shavings of volunteer 1 arranged in the approximate position of the corresponding hair root sections at the time of alcohol consumption. The hair root dimensions were taken from Vogt et al. [21] and Pötsch [24]. It follows that incorporation of EtG occurs to a high extent into the completed hair matrix in the suprabulbar and isthmus regions

melanin since incorporation of EtG was found not to depend on hair colour [45].

## Incorporation in hair from sweat

Incorporation from sweat is limited to the excretion time of EtG, in the present study about 22 h after the start of drinking. In longer hair, incorporation of substances can occur over an extended proximal length as far as the hair is wetted by sweating. Moreover, sebum lipids are partially degraded on the skin surface acting as an emulsifier. Therefore, EtG containing sweat can be trapped by sebum resulting in an emulsion that covers the skin surface and the hair and may lead to incorporation of EtG in more distant hair segments. This is different for shaved beard hair where only the newly grown stubbles are exposed to sweat. Since the first shaving was collected only about 14 h after the start of drinking, deposition of EtG from sweat could be the reason of the positive result on days 1 and 2 (Fig. 1). Inclusion of the infundibulum (length 0.58±0.08 mm [21]) which in principle is accessible for sweat could extend this by two more days. However, the infundibulum is filled with upward flowing lipophilic sebum which may create a physical barrier against EtG penetration into the hair. Altogether, a decrease of the concentration from day 1 to day 4 would be expected.

This is not in agreement with the experimental findings for volunteers 1 and 2 (Fig. 1) with relatively low concentrations in the shavings collected 14 h after the start of drinking, medium concentrations on day 2 and maximum concentrations on days 3 and 4. Concentrations after day 4 cannot be explained by sweat. Therefore, incorporation from sweat seems to be of minor importance in case of volunteers 1 and 2. Only for volunteer 3 that the higher concentrations in the shavings of days 1 and 2 point to a substantial contribution of sweat during the first 2 days, although the highest concentration would have been expected on day 1.

## Comparison with beard hair results from literature

The results of previous studies about the time course of drug concentrations in beard hair after single dose and the interpretation of the authors [12–18] are compared with the present investigation in Table 2. The time course was generally not interpreted in context of the anatomic dimensions of the hair root and of the growth rate, and the limited access of sweat to hair on daily shaved skin was also not considered.

Most of these drugs have a short half-life in the blood similar to EtG but with a more lipophilic and basic structure which favours binding to melanin. For most substances, the first appearance occurred on day 1 or 2 with a maximum concentration on day 1 (nicotine), day 2 (propyphenazone) or day 3 (methoxyphenamine and codeine). In these cases, sweat was interpreted as a contributing or even as the main way of incorporation. The

**Table 2** Results of present study in comparison to literature data about drugs in beard hair after single dosage

| Substance and dose | Number of volunteers | Half-life in blood | Time of detection after intake, day | | | Interpretation by the authors* | Reference |
|---|---|---|---|---|---|---|---|
| | | h | First | Highest | Last | | |
| Ethyl glucuronide | 3 | 1.7–3.1 | 1 | 2–4 days | 8–9 days | The predominant portion of EtG is incorporated from the blood in the upper part of the hair root between suprabulbar region and isthmus. Deposition from sweat was found to be of minor importance but could play a greater role in long hair. | Present study |
| Morphine 10 and 20 mg after 7 days; codeine 60 and 120 mg after 7 days | 2 | 1.3–6.7 (Morphine); 1.2–3.9 (Codeine) | 1 | 8 | ? | Early appearance by sweat, time lag between dose administration and first appearance in hair is approximately 7–8 days. *The time course remains unclear because of two subsequent administrations.* | Cone [12] |
| Methoxyphenamine, 50 mg | 6 | No data | 1 | 3 | 10–12 | It was estimated that the clearance of drugs from the hair matrix cells would take 5–8 days more than the duration of drug use. | Nakahara et al. [13] |
| Meprobamate 400, 800 and 1,200 mg | 16 | 6–17 | 4–5 | 7–9 | 12–14 | Time lag of approximately 4–5 days between the administration and appearance in hairs, probably growth time between the bulb area in the follicle and the skin surface. | Kintz et al. [15] |
| (Nicotine, 4 mg) Cotinine | 6 | 10–27 | 3 | 5 | 7 | *Nicotine was not detected.* The data indicate that the main route for incorporation of cotinine into hair is during hair growth. Transfer into beard from sweat is of little importance. | Gwent et al. [14] |
| Nicotine, 4 mg Cotinine | 6 | 0.4–1.2, 10–27 | 1 | 1 | 12 | Deposition of nicotine and cotinine from sweat onto the growing hair shaft was a contributing mechanism. | Bernert et al. [18] |
| Codeine, 60 mg | 6 | 1.2–3.9 | 1 | 3 | 14 | It is proposed that an early and the largest transfer of opiates into beard hair is through sweat. | Callaghan et al. [16] |
| Propyphenazone, 900 mg in 2 days | 1 | 1–3 | 2 | 2 | 4 | The results suggest that incorporation may be mainly due to excretion in sweat and subsequent incorporation into the hair. | Yegles and Wennig [17] |

*Details presented in italics are remarks of the authors of the present paper

**Table 3** Concentrations of EtG in beard hair after single excessive drinking

| Volunteer | EtG in hair, pg/mg | | |
|---|---|---|---|
| | Maximum (day) | Mean of days 1–32 | Related to 3 months |
| 1 | 184 (3) | 16.0 | 5.7 |
| 2 | 74 (4) | 8.6 | 3.1 |
| 3 | 242 (2) | 24.6 | 8.7 |

time course was similar to that of EtG (Fig. 1) showing that also for these substances redistribution from the lower parts of the follicle to blood should have occurred.

A different behaviour with incorporation exclusively in the hair root was described only for meprobamate [15]. The reason is not clear and may result from the special aliphatic structure of this compound with two carbamate groups.

### Interpretation of the EtG concentrations

The maximum concentrations measured between days 2 and 4 for the three volunteers after the single excessive drinking (74–242 pg/mg) are far above the cutoff of 30 pg/mg presently used for discrimination of alcohol abuse from social drinking. However, for comparison with routine data, the impact of these values on the mean concentration in the 0–3-cm hair segment must be seen. In Table 3, the mean concentrations of all shavings over the time period of the experiment (32 days) was calculated and related to 3 months (90 days). Comparison with the cutoff of 30 pg/mg for excessive drinking and 7 pg/mg for social drinking in the published or proposed consensuses of the Society of Hair Testing (http://www.soht.org/ (April 10, 2012)) shows that in a 1-cm proximal segment all three volunteers would be interpreted to be social drinkers, whereas in the 3-cm proximal segment, there would be evidence for alcohol consumption only for volunteer 3 (8.7 pg/mg) and the concentrations for volunteers 1 and 2 (5.7 and 3.1 pg/mg) would not be in contradiction to alleged abstinence. It can be expected that the real concentrations in the 3-cm proximal segment would be even lower because of wash-out effects.

## Conclusions

The results and the discussion lead to the conclusion that at least for daily shaved beard hair, incorporation of EtG from sweat is not the dominating route. Instead, EtG is mainly incorporated within the hair root with highest concentrations in the isthmus and in the upper part of the suprabulbar region. Diffusion from the blood through the surrounding tissues and the outer and inner root sheaths into the completed hair in these regions seems to contribute essentially to the final concentration in the hair. Nevertheless, deposition of EtG from sweat can play a more important role in longer hair where the hair shaft provides a larger area for deposition than the shaved hair stubble.

As a prerequisite for a time-resolved interpretation, it was found that the width of the EtG-positive band in beard hair after a single high alcohol dose corresponds to a time period of 8 days with more than 80 % within 4–5 days. It follows from the maximum concentrations of 74 to 242 pg/mg that a single excessive drinking can be well detected in the corresponding sufficiently short hair segment. However, a negative result can be expected in the routinely investigated 3-cm proximal scalp hair segment with high probability because of dilution by negative hair.

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