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THE DISTRIBUTION AND METABOLISM OF ACETALDEHYDE IN RATS DURING ETHANOL OXIDATION—I.

THE DISTRIBUTION OF ACETALDEHYDE IN LIVER, BRAIN, BLOOD AND BREATH

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Abstract—An *in vivo* investigation was made of the distribution of acetaldehyde (AcH) during ethanol metabolism. Different doses of ethanol were administered orally to male and female Sprague–Dawley rats ($n = 96$) and AcH measured at various times thereafter in the liver, blood, brain and breath. The results showed that the liver was the primary site for the oxidation of the ethanol-derived AcH. Only a small amount of the total AcH formed in this organ escaped into the rest of the body, but this amount increased with increased hepatic ethanol concentration. The hepatic AcH level was higher in male rats than in females with the same hepatic ethanol concentration. The extrahepatic AcH levels in arterial cerebral and in peripheral tail blood correlated well with the corresponding hepatic AcH levels. The bulk of the hepatic AcH output was eliminated extrahepatically, thus drastically changing the AcH level from that initially leaving the liver. Sex differences also appeared in the extrahepatic blood AcH levels, with the female rats displaying higher AcH levels, as a result of their less efficient extrahepatic AcH elimination. The peripheral tail blood AcH was found to be similar to the AcH level of the venous blood before the hepatic blood AcH is added to it. Regardless of the AcH levels in the liver and blood, no AcH was found in the brain. Less than 5 per cent of the hepatic AcH output was exhaled. Pentobarbital anaesthesia strongly depressed the amount of AcH exhaled. The AcH in the breath did not reflect the hepatic AcH as well as the blood AcH levels did.

Acetaldehyde (AcH), the first metabolite formed during ethanol oxidation, and its role in the actions of ethanol have recently become the subject of much interest. It has been shown with rat strains raised by genetic selection for high or low voluntary ethanol intake, that during ethanol oxidation those with a high ethanol preference have lower blood and liver levels of AcH than those with a low preference [1]. The parallels between differences in ethanol preference and AcH levels were in accordance with the assumption that AcH may interact with brain metabolism, and thus alter neural function and affect behaviour [2].

The pharmacological effects of AcH depend, of course, upon the quantities of AcH that leave the liver and reach the brain and other parts of the body. There has, however, been much confusion concerning the distribution of AcH during a normal ethanol oxidation. For instance, as can be seen in the review by Truitt and Walsh [2], the majority of the previous studies have measured the AcH levels occurring during disulfiram treatment and thus convey little information about the normal levels after ethanol administration. Among the remainder of the studies there still are great variations in the blood AcH levels reported. Similarly very discrepant values have also been reported for AcH levels in the brain [3–11]. The discrepancies in AcH values appear to be partly the result of different experimental conditions (animals, sexes, ethanol doses and times of ethanol oxidation) and partly because of technical difficulties. The main difficulty in this respect has been an ethanol-induced non-enzymatic formation of AcH in protein-precipitated blood or tissue samples [12–14].

Because of the current interest and the theoretical importance recently attached to AcH, it was felt that these discrepancies should be resolved. Consequently, an investigation was conducted to determine the AcH distribution in the liver, brain, blood and breath. In order to get a well-rounded picture under different conditions of ethanol oxidation, the AcH distribution was studied after various doses of ethanol and at different times after ethanol administration.

MATERIALS AND METHODS

Animals. Fed male and female Sprague–Dawley rats, 5–6 months of age were used. The 48 males weighed 386 ± 47 g (mean ± S.D.), while the 48 females averaged 256 ± 30 g. The rats were given a standard laboratory diet (Astra-Ewos AB, Södertälje, Sweden) and water *ad lib.*

Liver and brain freeze-stops. The experimental design is shown in Fig. 1. Liver and brain freeze-stops were made 15, 30, 60, 120 and 240 min after the administration of the different ethanol doses. Fifteen min prior to the freeze-stops, the rats were anaesthetized with 30 mg pentobarbital (Nembutal R, Abbot S. A., Brussels, Belgium) per kg body wt intraperitoneally (i.p.), given as a 1% (w/v) solution in saline.

Time \ Dose	0.75	1.5	3.0
0.25	1	4	8
0.50	2	5	9
1.0	3	6	10
2.0		7	11
4.0			12

Fig. 1. Experimental design for the ethanol dose (g/kg body wt) and the time between ethanol administration and sampling (h). The groups are numbered from 1 to 12 and each group includes 4 male and 4 female rats. Ethanol was given *per os* as a 15% (w/v) solution in saline.

A special operation table was constructed, which enabled the liver and brain freeze-stops to be taken at the same moment (by two persons). A 2–3 g sample of each liver was frozen *in situ* by means of aluminium clamps precooled in liquid nitrogen. The brains were freeze-stopped by means of the coppertube method previously described [10]. The frozen liver samples and brains were pulverized in mortars cooled with liquid nitrogen and the tissue powder was suspended in 0.6 M ice-cold perchloric acid, shaken, and the precipitates were centrifuged at 4000 *g* for 15 min at 4°. Ethanol and AcH were determined from the supernatants. In order to determine the blood contamination of the brain a small part of the brain powder was suspended in water and the hemoglobin was spectrophotometrically estimated by the method of Klein [15].

Cerebral blood determinations. Immediately after the brain was removed two blood samples were taken from the arterial blood spurting into the cerebral cavity. The first blood sample (0.1 ml), taken less than 5 sec after the freeze-clamping, was pipetted into 1.2 ml 0.6 M ice-cold perchloric acid, shaken, the precipitates centrifuged as before, and the ethanol and AcH measured. The second blood sample was hemolysed in water and used as a reference for the estimation of the blood contamination in the brain samples.

Peripheral blood determinations. Blood samples were taken from the tip of the tail 6 min before the freeze-stops, and also 6 min before each of the other sampling times seen in Fig. 1, if the freeze-stops were made 30 min or more after ethanol administration. The blood samples were treated in the same way as that described for the first cerebral blood sample.

Breath determinations. Beginning 14 min before each of the times shown in Fig. 1 the rats were placed in glass cylinders, and the exhaled AcH and ethanol collected by a method described by Forsander and Sekki [16]. Compressed air is blown through the cylinder and then bubbled through 10 ml cold water (4°). The rats remained in the cylinders for 8 min each time and the total amount of AcH and ethanol exhaled during these periods was estimated.

Analytical technique. Ethanol and AcH were measured with a Perkin-Elmer F 40 gas chromatograph (column: 15% polyethylene glycol on Celite 60/100 and column oven temperature: 75°) using FID and application of the head-space technique as described previously [14]. The samples to be analysed were pipetted into serum bottles, sealed with a rubber stopper, and incubated for 15 min at 65° in a sampling turntable, after which head-space samples automatically were taken by means of an electropneumatic dosing system. Since AcH is non-enzymatically formed in the presence of ethanol in the tissue and blood supernatants [12–14], ethanol and AcH were measured from supernatants containing thiourea (p.a., E. Merck AG, Darmstadt, Germany), which has been found to prevent this non-enzymatic formation of AcH [14, 17]. The blood and brain supernatants had 25 mM thiourea and the liver supernatants 45 mM thiourea added. This method measures the total AcH content without regard to possible binding [14].

RESULTS

The distribution of ethanol after oral administration. Figure 2 demonstrates the distribution of ethanol in liver, brain, cerebral and peripheral (tail) blood after oral administration of 0.75, 1.5 and 3.0 g ethanol/kg body wt. No significant sex differences were found; the ethanol values from both sexes were therefore combined. Liver, brain and cerebral blood ethanol levels did not differ significantly. However, the peripheral blood ethanol level was not as high as the other levels during the first hour after the oral administration. From the data shown in Fig. 2, an approximate ethanol oxidation rate of 12 μmole/100 g body wt per min or 3.5 μmole/g liver per min can be obtained by extrapolating the hepatic ethanol levels to the time when the 3 g/kg dose is completely oxidized.

It should be noted that all these ethanol levels (Fig. 2) were measured during the pentobarbital anaesthesia. However, the peripheral blood ethanol levels from unanaesthetized rats did not differ significantly from the respective levels of the anaesthetized animals.

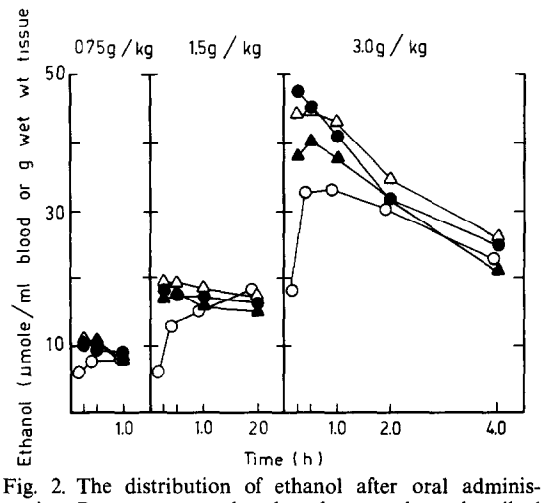

Fig. 2. The distribution of ethanol after oral administration. Rats were treated and analyses made as described in the Materials and Methods section. Liver (●), brain (▲), cerebral (△) and peripheral (○) blood ethanol values are given as means (S.D. = 10–30%) with the male and female rats combined. The number of animals per mean was 8 except in the following groups: brain, $n = 3$, group 1, Fig. 1; 5, group 3; 6, groups 4 and 5; 7, groups 2, 6 and 8, and cerebral blood, 3, group 1; 6, groups 4 and 5; 7, group 2.

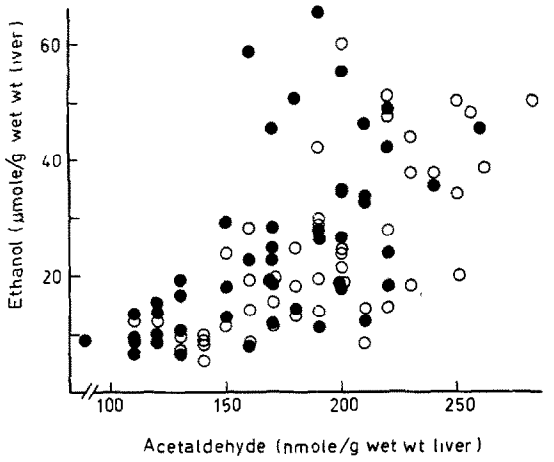

Fig. 3. Correlation between liver ethanol and acetaldehyde levels. Rats were treated and analyses made as described in the Materials and Methods section. Individual values for males are marked with open circles and those for females with solid circles.

Correlation between liver ethanol and acetaldehyde. The hepatic AcH level during ethanol oxidation was shown to correlate positively ($r = 0.68$, $p < 0.001$ for males and $r = 0.59$, $p < 0.001$ for females) with the hepatic ethanol concentration (Fig. 3). The shape of the correlation was curved and a significant ($t = 2.78$, $p < 0.01$ for all animals; $n = 96$) sex difference occurred, with the female rats displaying a lower level of AcH than the males at the same ethanol concentration. The average ethanol/AcH ratios ± S.D. after the dose of 0.75 g/kg ($n = 12$) were 67.0 ± 22.0 and 77.5 ± 18.1, after 1.5 g/kg ($n = 16$) 94.0 ± 19.0 and 103 ± 26 and after 3 g/kg ($n = 20$) 174 ± 43 and 203 ± 70 in male and female rats, respectively. No significant correlations were found when the hepatic AcH levels, within same ethanol concentration range, were plotted to the time after ethanol administration.

Correlation between liver and blood acetaldehyde. A nearly linear, positive correlation ($r = 0.81$ for males and $r = 0.59$ for females) was found between the liver and the cerebral blood AcH concentration (Fig. 4). A significant sex difference was demonstrated with the female rats having a higher AcH concentration in the cerebral blood compared with male animals having the same hepatic AcH levels.

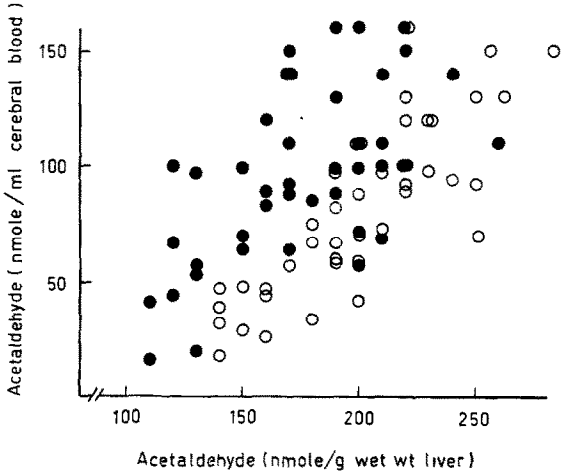

Fig. 4. Correlation between liver and cerebral blood acetaldehyde. Rats were treated and analyses made as described in the Materials and Methods section. Marking as in Fig. 3.

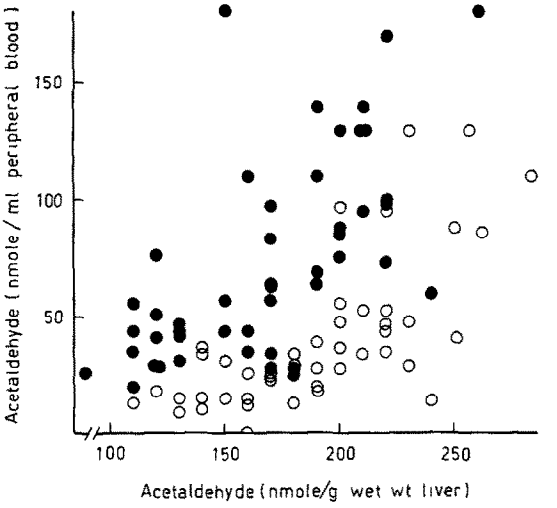

Fig. 5. Correlation between liver and peripheral (tail) blood acetaldehyde. Rats were treated and analyses made as described in the Materials and Methods section. Marking as in Fig. 3.

The same significant sex differences were observed in the peripheral blood as demonstrated in Fig. 5, where the (tail) blood AcH levels were plotted against liver AcH. The peripheral AcH levels were lower than the respective cerebral blood and liver levels, with a more distinct difference occurring in the male rats. Only slight differences were obtained in the peripheral blood AcH levels of anaesthetized rats compared to respective levels of unanaesthetized animals, with the female rats showing higher AcH levels during the anaesthesia (Fig. 6).

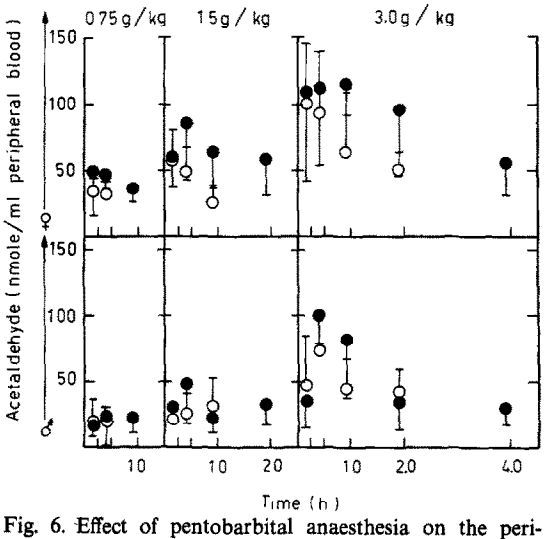

Fig. 6. Effect of pentobarbital anaesthesia on the peripheral (tail) blood acetaldehyde. Rats were treated and analyses made as described in the Materials and Methods section. (●) represents means − S.D. ($n = 4$) during pentobarbital anaesthesia. (○) represents means + S.D. ($n = 16$, group 8, Fig. 1; 12, groups 4 and 9; 8, groups 1, 5 and 10; 4, groups 2, 6 and 11) during no anaesthesia.

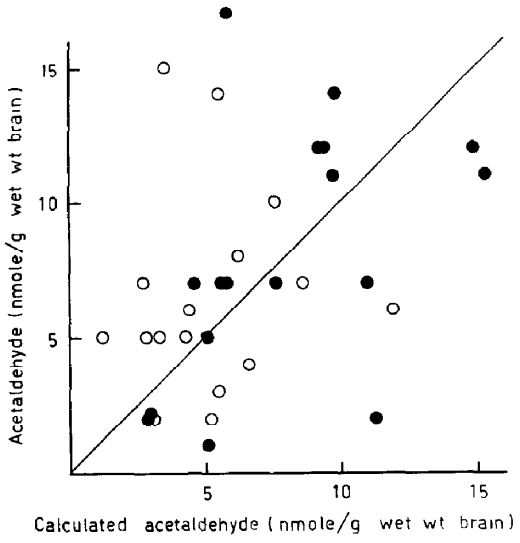

Fig. 7. Brain acetaldehyde during ethanol oxidation. Rats were treated and analyses made as described in the Materials and Methods section. Marking as in Fig. 3. The calculated acetaldehyde represents the expected acetaldehyde concentration caused by cerebral blood contamination in the freeze-stopped brain samples. The diagonal line represents a ratio of one between observed AcH in the brain and the amount of calculated AcH.

Brain acetaldehyde during ethanol oxidation. Only a small fraction of the hepatic AcH levels were found in the freeze-stopped brains. These samples contained an average contamination of 6.8 ± 2.7 ml of blood/100 g wet wt brain (mean $\pm$S.D., $n = 33$). Since this blood contained AcH which would be measured spuriously as being in the brain, it was necessary to calculate the amount of contaminating blood AcH in each sample. These are plotted in Fig. 7 against the total levels of AcH in each brain sample. The two sets of values were of the same magnitude; consequently, when the contaminating AcH was subtracted, essentially no AcH was found to be in the brain itself.

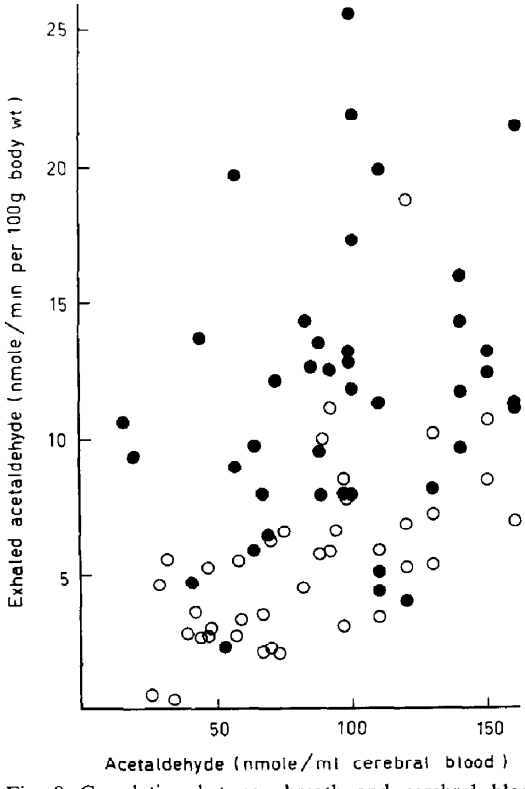

Fig. 8. Correlation between breath and cerebral blood acetaldehyde. Rats were treated and analyses made as described in the Materials and Methods section. Marking as in Fig. 3.

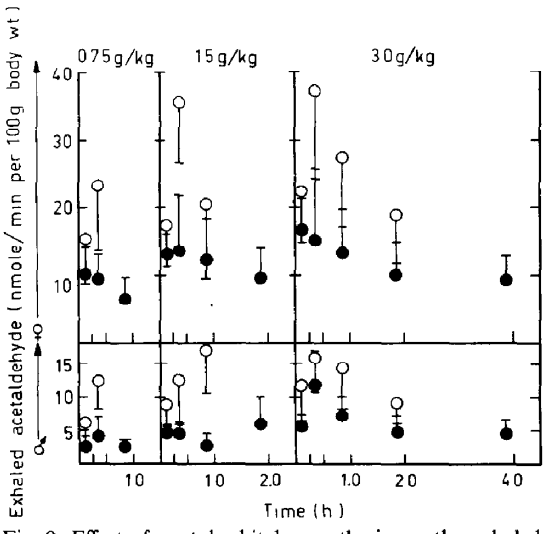

Fig. 9. Effect of pentobarbital anaesthesia on the exhaled acetaldehyde. Rats were treated and analyses made as described in the Materials and Methods section. (●) represents means + S.D. ($n = 4$) during pentobarbital anaesthesia. (○) represents means −S.D. ($n = 16$, group 8, Fig. 1; 12, groups 4 and 9; 8, groups 1, 5 and 10; 4, groups 2, 6 and 11) during no anaesthesia.

Breath acetaldehyde and ethanol. Breath AcH and ethanol were measured not as breath concentrations but as the total amounts exhaled during the 8 min periods. Figure 8 demonstrates the positive correlation between the amount of exhaled AcH and the cerebral blood AcH concentration ($r = 0.59$, $p < 0.001$ and $r = 0.24$, $p < 0.1$ for males and females respectively). The corresponding correlations between the exhaled and hepatic AcH were $r = 0.62$, $p < 0.001$ and $r = 0.30$, $p < 0.05$. When the AcH was calculated as amounts exhaled per body wt, significant sex differences occurred, with the female rats exhaling more AcH than the male animals. The average ratios between the amount of AcH exhaled (nmole/min per 100 g body wt) and the cerebral blood AcH concentration (nmole/ml) were $7.0 \times 10^{-2} \pm 3.9 \times 10^{-2}$ (mean $\pm$ S.D., $n = 39$) for males and $14.6 \times 10^{-2} \pm 11.9 \times 10^{-2}$ ($n = 41$) for females.

The correlation between the total amount of ethanol exhaled/time per body wt and the cerebral blood ethanol concentration was highly significant ($r = 0.76$, $p < 0.001$ and $r = 0.57$, $p < 0.001$ for males and females respectively). The same significant sex differences as with the AcH occurred, with the female rats exhaling more ethanol per body wt than did the male animals. The average ratios between the amount of ethanol exhaled (nmole/min per 100 g body wt) and the cerebral blood ethanol concentration (nmole/ml) were $6.8 \times 10^{-3} \pm 2.8 \times 10^{-3}$ ($n = 39$) and

$11.5 \times 10^{-3} \pm 5.6 \times 10^{-3}$ ($n = 41$) for males and females respectively.

The amounts of exhaled AcH and ethanol described above were determined from rats during pentobarbital anaesthesia. When not anaesthetized both male and female rats exhaled about twice as much AcH (Fig. 9). Anaesthesia had a similar effect, both qualitatively and quantitatively, on the exhaled ethanol.

DISCUSSION

Distribution of ethanol after oral administration. Gastric intubation was chosen as the means of ethanol administration because it corresponds best to the physiological conditions occurring when alcohol is drunk. The levels of ethanol found in the liver, cerebral blood and brain were all approximately the same. The level found in the tail blood was, however, initially lower, apparently because a longer time was required for the establishment of equilibrium there [18]. A similar effect has been seen previously and referred to as an "overshoot" in the brain ethanol level [19]. It may be responsible, in part, for the phenomenon of acute tolerance, i.e. the finding of a greater behavioural impairment during the ascending portion of the peripheral blood–ethanol curve than in the descending portion may partly be caused by the brain ethanol concentration being higher than the peripheral level during the ascending phase. Because tail-blood samples are so frequently used for the determination of ethanol, it is important that this discrepancy between the ethanol levels there and in the brain and liver be recognized.

Liver acetaldehyde during ethanol oxidation. Unlike the distribution of ethanol, the distribution of its first metabolic product, AcH, has until recently only been subjected to limited investigations. One of the main reasons for this has probably been difficulties with the determination of AcH from biologic samples [12, 20], including a non-enzymatic formation of AcH in the presence of ethanol in blood precipitates [12], in blood supernatants and, especially, in liver supernatants [13, 14]. Recent data from our laboratory has shown that this non-enzymatic production of AcH can be stopped by the use of thiourea [14, 17].

Hepatic AcH levels during ethanol metabolism have previously been reported by Forsander *et al.* [21], who measured the hepatic AcH in rats 60 min after an intraperitoneal ethanol injection, and by Tsukamoto [7], who determined hepatic AcH levels in guinea pigs at different times after orally administered ethanol. However, the problem of non-enzymatic AcH formation had not yet been recognized and, therefore, neither study controlled for it.

Subsequent *in vivo* studies have measured the AcH levels properly as well as the rate of ethanol oxidation [1, 22]. From these and an estimated hepatic blood flow of 0.79 ml/min per g liver [23], it can be calculated that over 95% of the AcH produced in the liver is further metabolized immediately. Perfusion studies have also indicated that the major portion of the ethanol-derived AcH is metabolized within the liver, and only a small amount leaves the liver [1, 24, 25]. The same conclusion can be obtained from the data collected in the present study. In addition the present results show that the amount of AcH escaping the liver correlates positively to the hepatic ethanol concentration (Fig. 3) and that the hepatic AcH metabolism achieves a steady state within 15 min of the administration of ethanol.

Blood acetaldehyde during ethanol oxidation. Very different levels of ethanol-induced AcH have been reported previously (see review by Truitt and Walsh [2]). Some of the differences appear to be legitimate consequences of different experimental conditions, such as whether pretreatment with disulfiram was employed, the species used, the metabolic state, the type of diet and whether there had been previous chronic alcohol administration. In other cases, however, the discrepancies can be traced to non-specific, insensitive or unreliable measurement techniques [20].

The present study points out another cause for differences: the location in the body from which AcH is sampled. The level in the liver is highest; of the locations sampled here, the cerebral blood was next highest, the peripheral blood from the tail came third, and the brain last.

The AcH concentration in the liver output via the *vena hepatica* has been shown to be approximately the same as that in the liver itself [21]. This blood is, however, diluted by other venous blood on its way to the heart. Consequently, the AcH concentration in the arterial blood could be written as:

$$[\mathrm{AcH}]_{\mathrm{arterial}} = (X\%[\mathrm{AcH}]_{\mathrm{hepatic}} + (100 - X)\%[\mathrm{AcH}]_{\mathrm{venous}}) \times k, \quad (1)$$

where X is the dilution of the hepatic blood, reported to be about 20% in rat [26], and k is a correction factor which includes any possible elimination of AcH from the blood before it becomes arterial by oxidation in the blood, by diffusion out of the blood or by elimination in the lungs. However, little AcH is thought to be oxidized within or diffused from the blood during the short interval needed for the blood to flow from the liver to the aorta. Some AcH is lost in the lungs, but, as will be discussed later, this represents less than 5 per cent of the hepatic output. Consequently little error is introduced if k is eliminated from equation (1). This equation can be rewritten as:

$$[\mathrm{AcH}]_{\mathrm{venous}} = 1.25[\mathrm{AcH}]_{\mathrm{arterial}} - 0.25[\mathrm{AcH}]_{\mathrm{hepatic}}, \quad (2)$$

and the values for the arterial and hepatic AcH levels shown in Fig. 4 can be introduced to calculate the approximate venous AcH concentration. The values calculated in the present study for the venous AcH levels are shown in Fig. 10, plotted against the hepatic levels. It will be noticed that the peripheral tail blood AcH concentrations in Fig. 5 present a rather similar picture. Therefore, measurements of the tail blood could be used to represent the venous AcH concentration.

The importance of extrahepatic factors in modifying the liver output AcH levels was also demonstrated in the sex differences revealed in this work: the male rats had a lower blood AcH level than the females, but had a higher level in the liver than the females.

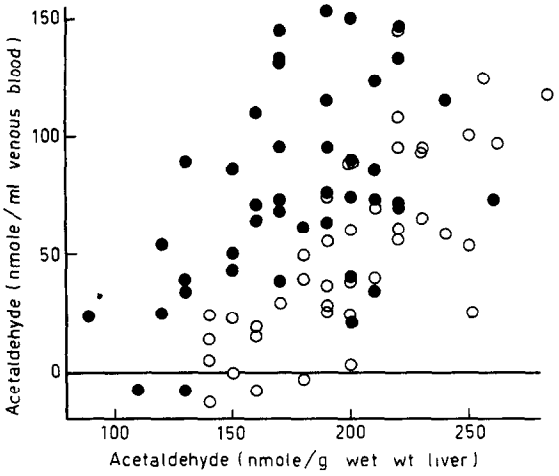

Fig. 10. Correlation between calculated venous blood and liver acetaldehyde. Rats were treated and analyses made as described in the Materials and Methods section. Marking as in Fig. 3. The venous blood acetaldehyde is calculated by equation (2).

Similar sex differences have previously been reported in the ANA (non-alcohol drinking) rat strain [1]. Another finding of the same sort was reported by Kesäniemi [27]; the peripheral blood AcH in pregnant rats was found to be higher than in non-pregnant rats with same hepatic AcH levels. The probable explanation for the sex differences is that the female rat may have a slower extrahepatic AcH elimination; a tendency which might even be potentiated during pregnancy. Theoretically differences in an extrahepatic AcH formation, blood flow rate or distribution volume could also have contributed to the sex differences obtained here.

Brain acetaldehyde during ethanol oxidation. The role of AcH in the actions of ethanol in the brain has recently become a topic of strong interest [2, 28]. Much of the discussion and speculation has centered on the condensation with the brain biogenic amines [29, 30] and interactions with their metabolism [2, 31]. Brain AcH levels, which could cause such effects, had been found in the rat [3–6], guinea pig [7] and mouse [8, 9, 11]. However, in a recent study Sippel found no AcH in rat brain after an intraperitoneal ethanol dose of 3 g/kg when the AcH concentration of the cerebral blood was less than 200 nmole/ml [10]. Only with higher cerebral blood AcH levels could measurable amounts of AcH be detected in the brain. In the present study the cerebral blood AcH concentration did not exceed 200 nmole/ml and no significant amounts of AcH could be found in the brain, thus supporting the previous findings by Sippel.

There appear to be two important differences, which could explain the discrepancies between the recent study by Sippel and our study on one hand and the other previously reported studies on the other hand. First we corrected the brain AcH values for AcH contained in the small amount of blood contamination in the brain samples. The second and more important difference was that we used thiourea to inhibit the non-enzymatic formation of AcH, which occurs in blood and liver supernatants [13, 14]. In preliminary studies, we found that this non-enzymatic formation of AcH did occur also in rat brain supernatants. The amount of AcH formed in the supernatants to which ethanol was added was of such an order that it easily could explain the earlier findings of high brain AcH levels, measured at corresponding conditions.

There are some factors which could, theoretically, have produced high brain AcH levels during ethanol oxidation. These include higher blood AcH concentration, ethanol metabolism in the brain and impaired oxidation of AcH in the brain or in the cerebral capillaries. It is also important to consider possible strain and species differences. In a study of brain AcH in mice by Ortiz *et al.* [9] no non-enzymatic AcH formation was reported with ethanol present. It, therefore, appears that mice might differ from rats in this respect.

The total absence of AcH in the rat brains strongly suggests that the AcH is metabolized rapidly before reaching the brain parenchyma. The cerebral capillary walls have been proposed to oxidize a certain amount of AcH, and, thus, act as an effective blood–brain barrier against exogenous AcH [10]. An alternative explanation would be that within the present AcH range most of the arterial cerebral blood AcH is bound in the erythrocytes and, therefore, not free to enter the brain parenchyma. This could be the case since the total AcH contents were measured without differentiating between bound and unbound AcH. Considering the role of AcH in the actions of ethanol in the brain, the absence of AcH levels in the brain is important to recognize. It means that reactions such as the condensation of AcH with the biogenic amines, which demands a relatively high AcH concentration [29], seem unlikely to occur within the brain. A more likely possibility would be that AcH could interfere with the metabolism of biogenic amine [31], by competing for the aldehyde dehydrogenase or reductase, which normally is used for the metabolic pathway of the biogenic amines. The present results suggest that this interaction would be unlikely to take place in the brain, but would be more likely in the cerebral capillary cells. Nevertheless, a role of an AcH metabolism inside the brain cannot be completely excluded because AcH could theoretically be formed and oxidized here without producing any measurable levels. The possibility of formation of AcH from ethanol within the brain was suggested by Raskin and Sokoloff [32] who demonstrated a small amount of alcohol dehydrogenase activity in the rat brain. Veloso *et al.* and Tabakoff and Wartburg [33, 34], however, calculated that ethanol oxidation rate in the brains would be negligible (in the range of 1 nmole/min per g brain).

Acetaldehyde in the breath during ethanol oxidation. Exhaled AcH during ethanol oxidation has been studied surprisingly little compared to its possible role in the elimination of the extrahepatic AcH. Freund and O'Hollaren [35] have measured AcH in the breath of man, Redmond and Cohen [36] in that of mice and Forsander and Sekki [16] in rats. None of these investigators have tried to make any estimations on how much of the ethanol-derived AcH is exhaled.

From the calculated value of ethanol oxidation rate (see the Results section) and from the data of Fig. 9, it can be calculated that the amount of exhaled AcH was 0.02–0.10 per cent (for males) and 0.05–0.25

per cent (for females) of the total amount of AcH formed during ethanol oxidation. The corresponding percentages for the exhaled ethanol were 0.4–5.0 per cent and 0.5–6.7 per cent for males and females respectively. The exhaled AcH amounted to approximately 0.4–2.0 per cent (males) and 1–5 per cent (females) of the hepatic AcH output (about 5 per cent of the hepatic AcH formation), thus showing that only minor amounts of the extrahepatic AcH are exhaled. Pentobarbital anaesthesia was found to reduce strongly the amount of exhaled AcH and ethanol.

Sex differences in the opposite direction, with male mice exhaling during ethanol oxidation several times more AcH than females of the same strain, have been found by Redmond and Cohen [36]. However, the conditional variables, such as the distribution of AcH or ethanol, were not determined and thus it is impossible to tell whether the sex differences were caused by hepatic or extrahepatic differences, which makes it difficult to compare these mouse results to our rat results.

Freund and O'Hollaren suggested from their human data that the alveolar air AcH concentration rapidly reaches a plateau due to a constant rate of ethanol oxidation in the liver [35]. Forsander and Sekki have recently shown that the exhaled AcH correlates positively with the exhaled ethanol [16]. This was confirmed in the data obtained here, demonstrating that the amount of AcH in the breath is determined by the hepatic output of AcH which is, in turn, determined by the hepatic ethanol concentration, and modified by extrahepatic AcH elimination. Thus the high initial levels of exhaled AcH found by Forsander and Sekki [16] were probably caused by a corresponding high hepatic ethanol level and the resulting high AcH output.

The breath AcH has been assumed to reflect more reliably the hepatic AcH concentration than the peripheral blood AcH [16, 35]. Furthermore, many of the technical problems involved in blood or tissue AcH determinations are eliminated. Thus it was surprising to find such a weak correlation between the expired AcH and the arterial cerebral blood or hepatic AcH in spite of the very high correlation between the ethanol values. This shows that there are certain limitations in using breath measurements for getting a reliable picture of the AcH levels within the body.

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