PRO EXPERIMENTIS

Determination of acetaldehyde in human blood[1]

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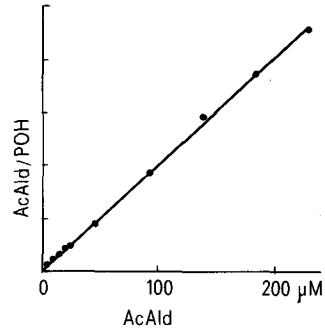
Summary. A method for the determination of acetaldehyde in human plasma by headspace gas chromatography is described. Chloralhydrate, an inhibitor of aldehyde dehydrogenase, is immediately added to the blood sample to prevent a rapid disappearance of acetaldehyde in the erythrocytes.

In the last few years evidence has accumulated showing the importance of acetaldehyde as a major toxic metabolite in the effects of alcohol. However, research in this area has been rendered difficult by many problems in determining acetaldehyde in biological samples. Older colorimetric, spectrophotometric, radiochemical and enzymatic methods lacked specificity and sensitivity and have mostly been replaced by gas chromatographic techniques, especially the headspace technique[3]. Although gas chromatographic methods are specific, sensitive and rapid, they suffer from the difficulty of preserving the original acetaldehyde in the sample. Levels can be elevated during the determination procedure due to artificial formation of acetaldehyde, if ethanol containing samples are deproteinized by precipitation with perchloric acid[4]. As a preventive measure the use of thiourea has been suggested[5,6], but this was also found to be unsatisfactory, especially for blood[7]. This problem of the non-enzymatic formation of acetaldehyde is still unsolved, and is aggravated by the fact that it varies with the ethanol concentration and the blood dilution[8-10]. An extensive review of the subject has recently been published[11]. On the other hand the original acetaldehyde level can be diminished because of a fast disappearance of the acetaldehyde from venous blood samples after termination of the sampling[12]. The presence of aldehyde dehydrogenase in human erythrocytes[13,14], and their capacity to metabolize acetaldehyde to acetate[15], have been demonstrated. In view of these findings, the previously reported absolute levels of acetaldehyde in human blood after alcohol ingestion must be considered with great caution. Therefore, we have developed a new method for the determination of acetaldehyde in human plasma which avoids all these problems.

In a first series of experiments acetaldehyde was added to venous blood immediately after sample collection and incubated at 37 °C for varying periods of time before precipitation with perchloric acid. Acetaldehyde was then determined by the headspace gas chromatographic technique described below. As shown in the table, about 50% of the acetaldehyde disappeared within 5 min, thus confirming previous findings[12,15]. Similar results were obtained with a suspension of erythrocytes in physiological saline, indicating that the erythrocytes were responsible for the removal of acetaldehyde. This interpretation was substantiated by a recovery of about 95% if acetaldehyde was added to human plasma. The recovery, however, was not complete, indicating that a small percentage of the acetaldehyde binds to plasma proteins. This binding is appearently not reversed by perchloric acid precipitation and consequent equilibration with the vapor phase at 60 °C for 30 min. With erythrocytes or whole blood the presence of ethanol (0.1-0.5 g/l) led to the artificial formation of variable amounts of acetaldehyde whereas no such production of acetaldehyde was found with plasma alone.

Based on these findings we attempted to determine acetaldehyde in plasma instead of whole blood, thus avoiding the problem of the non-enzymatic formation of acetaldehyde from ethanol. Since up to 30% of acetaldehyde added to human blood were already lost after 90 sec, this rapid removal had to be stopped for the time required to prepare plasma by centrifugation. Assuming that metabolism by aldehyde dehydrogenase of the erythrocytes was the cause of the disappearance of the acetaldehyde, several inhibitors of this enzyme such as disulfiram, calcium carbimide, 4-nitroacetophenone and chloralhydrate were added to the blood simultaneously with the acetaldehyde. Of all the inhibitors tested, 50 mM chloralhydrate proved to be most efficient in preventing the disappearance of acetaldehyde. With this procedure, if acetaldehyde was added to whole blood, the recovery in plasma gained after centrifugation was over 90% as compared to the recovery obtained for acetaldehyde added directly to plasma. The addition of 0.5 g/l ethanol did not interfere with this procedure, confirming that no artificial acetaldehyde was formed in the absence of hemoglobin. The precipitation of the plasma, however, was necessary, because the yield was only about 70% without this step, presumably due to acid reversible binding of the acetaldehyde to plasma proteins. Chloralhydrate reversed this binding only partially, probably by competing for some of the binding sites of the



Standard curve for acetaldehyde (AcAld) in human plasma with 50 mM chloralhydrate, and with 1-propanol (POH) as internal standard. Peak area ratio (AcAld/POH) are plotted versus the acetaldehyde concentration.

Recovery of acetaldehyde under various experimental conditions. Acetaldehyde was added to the samples and incubated for 5 min at 37 °C. The remaining acetaldehyde was determined by the headspace gas chromatographic technique as described in the text. The values represent the mean ± SD of 4-11 determinations

Experimental condition	Acetaldehyde concentration (µM)	Residual acetaldehyde (% of control)
0.9% NaCl (control)	100	100
Whole blood	100	48.6 ± 5.3
Erythrocytes in 0.9% NaCl	100	53.1 ± 6.9
Plasma	50	95.6 ± 2.7
Whole blood + chloralhydrate	50	90.7 ± 3.5
Plasma without acid precipitation	50	69.5 ± 2.7

proteins for acetaldehyde. As shown in the figure this procedure allows the determination of acetaldehyde in plasma in a range from 1 to 200 μM.

On the basis of these results the following procedure was adopted to determine acetaldehyde in human blood. 2 ml of blood were collected from the cubital vein with a butterfly needle with 3 inches of tubing, allowing the blood to drop into a graduated 5-ml centrifuge tube containing 2 ml 100 mM chloralhydrate in 0.9% NaCl with 100 U-USP heparin. After mixing the plasma was isolated in the covered tube by centrifugation. 2 ml of the plasma were transferred into glass vials (total volume of 26 ml) containing 0.1 ml 60% perchloric acid and 1.0 ml internal standard solution containing 0.1 mg 1-propanol. The vial was immediately sealed with a rubber membrane and equilibrated at 60 °C for 30 min in a water bath. 5 ml of the gas phase were injected into a Perkin-Elmer 900 gas chromatograph equipped with a flame ionization detector and an Autolab Minigrator from Spectra-Physics. A glass column (1.8 m × 2 mm inner diameter) packed with Porapak Q 80-100 mesh was used. The operating conditions were: injector temperature 180 °C; column temperature 150 °C; detector temperature 290 °C; carrier gas: 30 ml/min of nitrogen. The retention times for acetaldehyde, ethanol and 1-propanol were 97, 151 and 383 sec. Samples of precipitated plasma in the sealed vials were stable at room temperature for 24 h.

1 This work was supported by the Swiss National Science Foundation.
2 The authors thank Miss Ch. Coullery and Miss E. Ernst for their technical assistance.
3 G. Duritz and E.B. Truitt, J. Stud. Alc. *25*, 498 (1964).
4 H.W. Sippel, Acta chem. scand. *27*, 541 (1973).
5 H.W. Sippel, Acta chem. scand. *26*, 3398 (1972).
6 J.F. Brien and C.W. Loomis, Clin. chim. Acta *87*, 175 (1978).
7 C.J.P. Eriksson, H.W. Sippel and O.A. Forsander, Analyt. Biochem. *80*, 116 (1977).
8 E.B. Truitt, J. Stud. Alc. *31*, 1 (1970).
9 C.J.P. Eriksson, H.W. Sippel and O.A. Forsander, in: The role of acetaldehyde in the actions of ethanol, vol. 23, p. 9. Ed. K.O. Lindros and C.J.P. Eriksson. The Finnish Foundation for Alcohol Studies, Helsinki 1975.
10 A.R. Stowell, R.M. Greenway and R.D. Batt, Biochem. Med. *18*, 392 (1977).
11 K.O. Lindros, in: Research Advances in Alcohol and Drug Problems, vol. 1, p. 111. Ed. Y. Israel, F.B. Glaser, H. Kalant, R.E. Popham, W. Schmidt and R.G. Smart. Plenum Publ., New York 1978.
12 C.J.P. Eriksson, M.E. Hillbom and A. Sovijärvi, Drug Alc. Dep. *4*, 148 (1979).
13 R. Pietruszko and R.C. Vallari, FEBS Letters *92*, 89 (1978).
14 K. Inoue, Y. Ohbora and K. Yamasura, Life Sci. *23*, 179 (1978).
15 A.R. Stowell, R.M. Greenway and R.D. Batt, Biochem. Med. *20*, 167 (1978).

The collagen substratum influences in vitro hatching and attachment of the mouse blastocyst in a serumless medium

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Summary. When a serumless medium is used for the in vitro growth and development of post-blastocyst mouse embryos, a collagen substratum causes a delay in the hatching from the zona pellucida. However, the collagen substratum is essential for blastocyst attachment and trophoblast cell outgrowth after hatching has taken place.

The in vitro growth and development of the post-blastocyst mouse embryo to the egg cylinder stage has been described previously[3-6]. The influence of culture media[7,8], amino acids[9], glucose[10,11], and nucleosides[12] on this stage of mammalian differentiation has been studied. All of these studies have utilized a serum component as part of the culture medium. Although information is available concerning the influence of a collagen substratum on hatching and attachment in serum supplemented media[13], little information is available utilizing a serumless medium. The objective of this study was to compare a collagen to a plastic substratum with respect to the in vitro hatching and attachment of the mouse blastocyst in a serumless medium.

Superovulation was induced in random bred Swiss mice by the method of Gates[14]. Injected female mice were caged overnight with male mice and mating was verified the following morning by the presence of a copulatory plug in the vagina. Ovulation was assumed to occur approximately 12 h after the HCG injection[15]. 4 days postmating blastocysts were recovered by flushing the excised uterus with 0.5 ml of modified Brinster's medium[16]. Recovered blastocysts were pooled in culture medium under silicone oil in an atmosphere of 95% air plus 5% CO_2. Previously sterile 60 × 30 mm plastic petri dishes were either layered with collagen reconstituted from rat tails as described by Hsu et al.[17], or used without the collagen layer. Individual petri dishes were equilibrated with 5 ml of the culture medium 60 min prior to the introduction of the mouse blastocysts. Previously pooled blastocysts were randomly assigned to either collagen or plastic substratum with Eagle's basal medium (BME) supplemented with 4% bovine serum albumin (BSA). Individual petri dishes were examined at times which corresponded to 130, 175 and 185 h post-ovulation.

Influence of a collagen substratum on postblastocyst embryo development in a serumless medium*

Substratum	Hours post-ovulation	Hatching from the zona pellucida (%)	Attachment to the substratum (%)
Plastic	130	44/100 (44)[a]	0/44 (0)[a]
	175	69/100 (69)[b]	4/69 (6)[a]
	185	69/100 (69)[b]	0/69 (0)[a]
Collagen	130	14/52 (27)[a]	7/14 (50)[a]
	175	33/52 (64)[b]	27/33 (82)[b]
	185	33/52 (64)[b]	27/33 (82)[b]

*Embryos cultured in Eagle's basal medium (BME) supplemented with 4% bovine serum albumin. Each time was replicated 3–5 times. Percentages with different superscripts are significant at the $p < 0.05$ level using a statistical analysis which tests a hypothesis between population proportions.